EFFECT OF ACUTE AND CHRONIC PRESSURE-THRESHOLD INSPIRATORY MUSCLE TRAINING UPON UPPER AND LOWER AIRWAY FUNCTION

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by

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ABSTRACT

There is evidence to suggest that inspiratory muscle training (IMT) may influence the functional properties of the muscles of the upper (UA) and lower (LA) airway. However, the nature and functional relevance of this influence is currently unclear. This thesis examined the effect of acute and chronic IMT in the context of UA and LA function. The ability of IMT to activate the UA dilator muscles, genioglossus (GG) and geniohyoid (GH), was examined using magnetic resonance imaging (MRI), as was the effect of chronic training on these muscles. In addition, the effect of acute and chronic IMT upon LA resistance (R_{rs}) and function was investigated in people with asthma using the Forced Oscillation Technique and conventional spirometry. For the UA, an acute bout of IMT at 60% maximal inspiratory mouth pressure (MIP) resulted in significant GG and GH activation ($P < 0.001$) as demonstrated by increases in the transverse relaxation time of muscle water (T_2). Despite this, MRI was unable to detect any effect of chronic IMT upon UA function. For the LA, the usual increase in R_{rs}, following deep inhalation (DI) in people with asthma was attenuated with both single and multiple breaths against a pressure-threshold load equal to 50% MIP. However, six weeks IMT had no effect on baseline airway function or response to DI. In conclusion, an acute effect of pressure-threshold IMT upon UA and LA function was demonstrated. A strong rationale for a beneficial influence of chronic pressure-threshold IMT was therefore demonstrated. However, the data were insufficient to either reject, or accept the hypothesis that IMT exerts more than a transient influence upon UA and LA function, but insights are presented that support the need for further investigations.

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LIST OF SYMBOLS AND ABBREVIATIONS

AHI	Apnoea-Hypopnoea Index
ASM	Airway Smooth Muscle
BTPS	Body Temperature and Pressure, Saturated with Water Vapour
CO_2	Carbon Dioxide
COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
CV	Coefficient of Variation
CWS	Chest Wall Strapping
DI	Deep Inhalation
EILV	End Inspiratory Lung Volume
EMG	Electromyography
FEF	Forced Expiratory Flow
F_ENO	Fraction of Expired Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second
FIF	Forced Inspiratory Flow
FOT	Forced Oscillation Technique
FRC	Functional Residual Capacity
F_{res}	Resonance Frequency
FVC	Forced Vital Capacity
G_{aw}	Airway Conductance
GG	Genioglossus
GH	Geniohyoid
Hz	Hertz
IMT	Inspiratory Muscle Training
IRL	Inspiratory Resistive Loading
LA	Lower Airway
MEFV	Maximal Expiatory Flow Volume
MEP	Maximum Expiratory Mouth Pressure
MHC	Myosin Heavy Chain
MIF	Mid Inspiratory Flow
MIP	Maximum Inspiratory Mouth Pressure
MRI	Magnetic Resonance Imaging
MVC	Maximal Voluntary Contraction
NANC	Non-Adrenergic, Non-Cholinergic
NDM	Nasal Dilator Muscle
NO	Nitric Oxide
OSAS	Obstructive Sleep Apnoea Syndrome
$P_{0.1}$	Mouth Occlusion Pressure at 0.1 s
P_{ao}	Airway Opening Pressure
PC_{20}	Provocative Concentration Causing a 20% Fall in FEV_1
PCA	Posterior Crico-arytenoid
PCO_2	Partial Pressure of Carbon Dioxide
P_{crit}	Critical Closing Pressure
PEF	Peak Expiratory Flow
$P_{ET}CO_2$	Partial Pressure of End-Tidal Carbon Dioxide
PIF	Peak Inspiratory Flow

PVFM	Paradoxical Vocal Fold Motion
R_{aw}	Airway Resistance
RPE	Rating of Perceived Exertion
R_{rs}	Respiratory System Resistance
RV	Residual Volume
SD	Standard Deviation
T	Tesla
T_2	Transverse Relaxation Time of Muscle Water
TLC	Total Lung Capacity
UA	Upper Airway
VC	Vital Capacity
VIP	Vasoactive Intestinal Peptide
V_T	Tidal Volume
X_{rs}	Respiratory System Reactance
Z_{rs}	Respiratory System Impedance

PEER REVIEWED ARTICLES AND CONFERENCE PRESENTATIONS

PUBLISHED DURING THE PREPARATION OF THIS THESIS

PUBLISHED FULL PAPERS:

How, S.C., Romer, L.M., & McConnell, A.K. (2009). Acute effects of inspiratory pressure-threshold loading upon airway resistance in people with asthma. *Respiratory Physiology and Neurobiology*. **166,** 159-163

How, S.C., McConnell, A.K., Taylor, B.J., & Romer, L.M. (2007). Acute and chronic responses of the upper airway to inspiratory loading in healthy awake humans: an MRI study. *Respiratory Physiology and Neurobiology*. **157**, 270-280

Taylor, B.J., **How, S.C.**, & Romer, L.M. (2006). Exercise-induced abdominal muscle fatigue in healthy humans. *Journal of Applied Physiology*. **100**, 1554-1562

CONFERENCE PRESENTATIONS:

Taylor, B.J., **How, S.C.**, & Romer, L.M. (2009). Severity of expiratory muscle fatigue is greater after whole-body exercise versus maximal voluntary hyperpnoea. *Medicine and Science in Sport and Exercise*. **30**, S479

How, S.C., Romer, L.M., Taylor, B.J., & McConnell, A.K. (2007). The acute effect of loaded inspirations upon airway resistance in mild to moderate asthmatics. *European Respiratory Journal*. **30**, 258s

How, S.C., Taylor, B.J., McConnell, A.K., & Romer, L.M. (2006). Increased activation of lingual muscles in response to acute inspiratory pressure-threshold loading in healthy humans: an MRI study. *European Respiratory Journal*. **28**, 356s

How, S.C., Taylor, B.J., McConnell, A.K., & Romer, L.M. (2006). Morphological changes of the upper airway in response to acute and chronic inspiratory loading in healthy awake humans: an MRI study. *European Respiratory Journal*. **28**, 420s

Taylor, B.J., **How, S.C.**, & Romer, L.M. (2005). Exercise-induced expiratory muscle fatigue in healthy humans. *Medicine and Science in Sports and Exercise*. **38**, S381

CHAPTER ONE

INTRODUCTION

1.1. OVERVIEW

The muscles of the upper (UA) and lower airway (LA) demonstrate a degree of plasticity in response to various stimuli. In the case of the UA, the muscles show an adaptive response to chronic endurance training in rats (Vincent *et al.*, 2002). Also, training the genioglossus (GG) by electrical neurostimulation has produced improvements in the incidence of snoring in humans (Randerath *et al.*, 2004). More recently Guimaraes *et al.* (2009) demonstrated a 39% reduction in the apnoea-hypopnoea index (AHI; an objective measure of obstructive sleep apnoea syndrome (OSAS) severity) after 3 months of oropharyngeal exercise.

The smooth muscle of the LA also responds acutely and chronically to different stimuli; in particular, to stretch. For example, it has been known for some time that a healthy individual has the ability to overcome pharmacologically induced bronchoconstriction by performing a deep inhalation (DI) to total lung capacity (TLC; Nadel & Tierney, 1961). Further, it is also known that the ability to reverse a bronchoconstriction is diminished in patients with asthma (Fish *et al.*, 1977). Airway smooth muscle (ASM) that is stiffer and less responsive to stretch induced by higher lung volumes, is thought to be an important factor in the differential response to DI seen in individuals with asthma (Fredberg *et al.*, 1997). One explanation for the response in asthma is that the ASM has adapted to a shortened state that is resistant to DI (Wang & Pare, 2003). Gunst *et al.* (1988) proposed that narrower and stiffer airways may require the application of a load in excess of that applied by a DI in order to dilate. Support for this has been provided in studies that have differentiated the effect of volume and pressure on the airway with chest

wall strapping (CWS). The authors concluded that the response to DI was mostly due to increases in transluminal airway pressure, rather than changes in lung volume (Duggan *et al.*, 1990). Further, it has recently been suggested that the reversal of airway obstruction by positive pressure inflation may be due to the application of a greater stretching force applied to the airway (Slats *et al.*, 2008).

Pressure-threshold inspiratory muscle training (IMT) applies a quantifiable transluminal pressure gradient across both the extra- and intra-thoracic airways. The effect of this pressure gradient upon UA and LA muscles is unknown, but increased activation of the UA muscles is known to occur during exercise (Williams *et al.*, 2000), when transluminal airway pressure is also increased. It may be that, via a similar mechanism, IMT is also able to activate the muscles of the UA and induce a training response. The effect of IMT upon the LA is also unknown, but the intrathoracic decompression, and increase in transluminal pressure produced by breathing against an inspiratory pressure-threshold load, may have a stretching influence upon the LA. It is possible that this stretching and subsequent remodelling of ASM may provide an explanation for improvements seen in forced expiratory flows reported by some post IMT (Weiner *et al.*, 1992; Lima *et al.*, 2008).

In view of the lack of knowledge relating to UA and LA responses to acute and chronic IMT, the primary aim of this thesis is to determine the acute and chronic influence of IMT on upper and lower airway function. In doing so, the focus of the thesis is upon the mechanical properties of the structures involved.

1.2. FORM AND FUNCTION OF THE AIRWAYS

The airways can be sub-divided into two major categories, according to their anatomic location; the location of the airways also has a strong influence upon their functional properties. The extra-thoracic, or upper airway (UA), consists of organs that are external to the thoracic cavity, whereas the organs of the intra-thoracic, or lower airway (LA), are located almost entirely within the thoracic cavity. The upper respiratory tract consists of the nose, nasopharynx, oropharynx, laryngopharynx, and larynx, whilst the lower respiratory tract consists of the trachea, all generations of the bronchial tree, and the lung parenchyma. These different anatomical locations result in the UA and LA being subjected to very different pressure gradients during breathing manoeuvres. Furthermore, the UA is involved in a range of non-respiratory functions (see below). Accordingly, the UA and LA have very different structural characteristics.

1.2.1. Structure of the Upper Airway

The structures of the UA are involved in functions including speech, deglutition, and breathing and act to modify and condition ambient air before it enters the lungs. The UA represents a necessary route for normal breathing and the structures within it account for approximately half of the resistance to airflow in the respiratory system (Ferris *et al.*, 1964). Approximately 20 muscles actively modulate the dimensions of the UA by constriction or dilation of the UA lumen (Fouke *et al.*, 1986).

From an embryological, morphological, and functional perspective, the muscles of the pharyngeal UA are considered to be skeletal (van Lunteren & Strohl, 1988). To appreciate the functional role of the UA muscles, an understanding of their anatomy and structure is required. The first part of this review will focus on the functional anatomy of the muscles of the UA. The focus will primarily be on the pharyngeal constrictors and dilators, as it is these muscles that are particularly relevant to the maintenance of UA patency. Some consideration will also be given to muscles of the larynx that control the position of the vocal folds (cords).

This section will review the literature in the context of the subsequent chapters of the thesis. In this regard the review is not intended to be exhaustive.

Muscles of the pharynx

The superior, inferior, and middle constrictor muscles form the postero-lateral wall of the pharynx and primarily function to aid deglutition via a peristaltic and sphincteric action (Figure 1.1; Series, 2002). Further, these muscles are also activated spontaneously in-phase with the respiratory cycle, contributing to the modulation of expiratory airflow resistance (Collett *et al.*, 1986). Neural innervation of all three pharyngeal constrictors is supplied by the pharyngeal plexus.

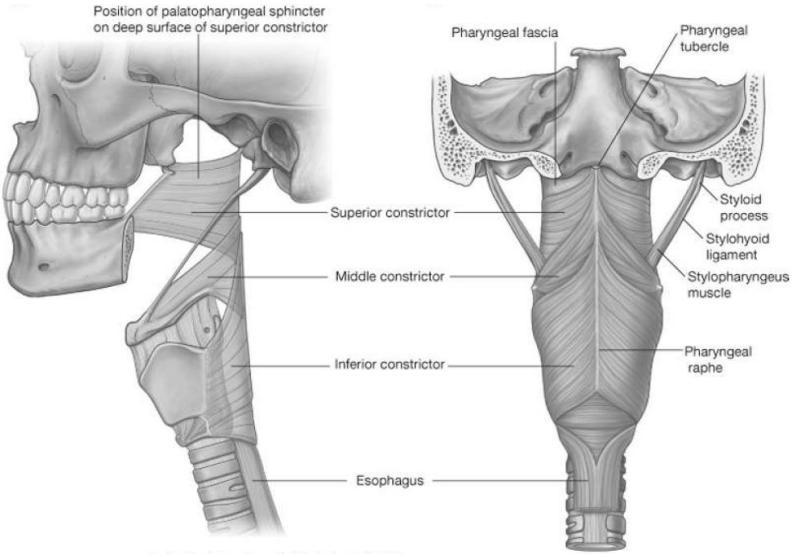

Figure 1.1. Anatomical arrangement of the pharyngeal constrictor muscles. From Drake *et al.* (2010).

A number of structures serve as the origin of the superior pharyngeal constrictor muscle. These structures include the side of the tongue, pterygomandibular ligament, and posterior border of the pterygoid plate, the pterygoid hamulus, and the posterior end of the mylohyoid line on the mandible. After curving around the pharynx, the fibres insert into the pharyngeal tubercle of the occipital bone and the median fibrous raphe on the posterior pharyngeal wall. The thyroid and cricoid cartilages, and the tendinous band that runs between them, form the origin for the inferior pharyngeal constrictor. Its fibres run toward the midline both horizontally and vertically to insert into the median fibrous raphe on the posterior pharynx, where there is large overlap with the other constrictor muscles.

In contrast, the pharyngeal dilator muscles are located predominantly anteriorly and laterally. The anterior wall of the oropharynx is formed by the posterior one-third of the tongue, whilst the anterior wall of the laryngopharynx is formed by

the muscles that act to control the position of the hyoid bone (Figure 1.2). The intrinsic muscles of the tongue consist of longitudinal, vertical, and transverse fibres that are confined to the tongue in the absence of any bony attachments. The primary role of the intrinsic tongue muscles is to act to alter the shape of the tongue.

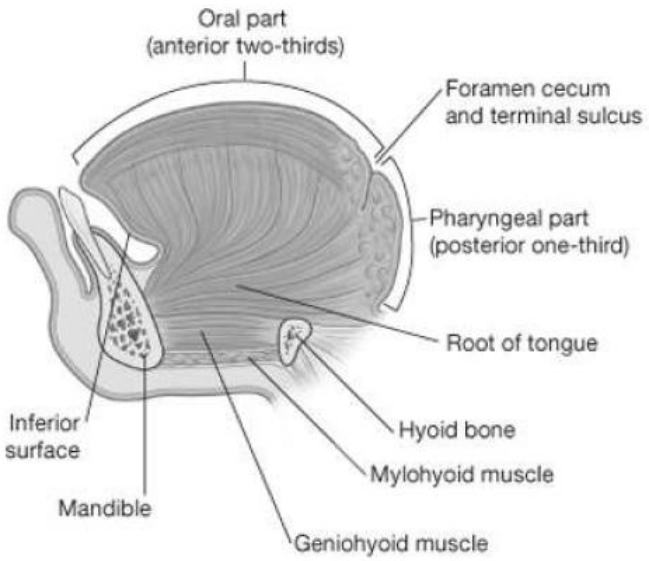

Figure 1.2. Paramedian sagittal section of the tongue showing the position of the genioglossus, geniohyoid, and the posterior one-third that forms the anterior wall of the pharynx. From Drake *et al.* (2010).

The extrinsic muscles of the tongue (Figures 1.2 and 1.3) include the genioglossus, hypoglossus, styloglossus, and palatoglossus. These muscles act to suspend the tongue between the stylous process of the basicranium, the mandible, the hyoid bone, and the soft palate. The genioglossus originates on the anterior portion of the mandible extending back into the tongue in a large fan-like shape. The genioglossus muscle is the primary extrinsic muscle of the tongue and acts to protrude, and along with the hypoglossus, also acts to depress the tongue. The styloglossus raises the tongue upward and back with the palatoglossus pulling

upwards the posterior aspect of the tongue. The palatoglossus is innervated by a branch of the vagus nerve (the pharyngeal plexus) with all of the other tongue muscle innervated by the hypoglossal nerve. The geniohyoid (GH), also innervated by the hypoglossal nerve, is a paired, narrow muscle that originates either side of the midline from the inferior mental spines on the back of the mandibular symphysis. It runs backward and downward inserting on the anterior surface of the body of the hyoid bone. GH activation elevates the hyoid bone and the base of the tongue causing the pharynx to dilate and the larynx to move up and forward during deglutition.

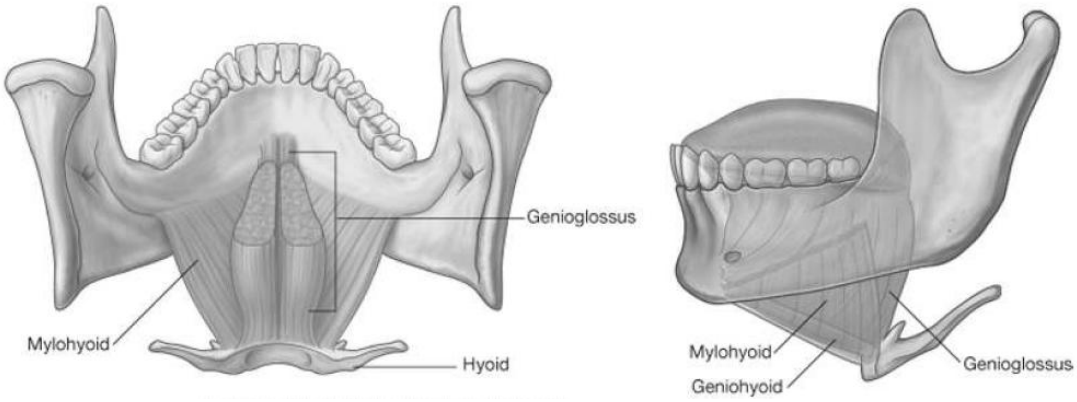

Figure 1.3. Anatomical arrangement of the pharyngeal dilator muscles. From Drake *et al.* (2010).

Muscles of the larynx

The laryngeal muscles are considered to be either extrinsic or intrinsic to the vocal folds. The extrinsic muscles act to assist with swallowing by changing the position of the thyroid cartilage in addition to minimising glottis displacement during breathing (van Lunteren & Strohl, 1988). Three infrahyoid depressors or 'strap muscles' (sternothyroid, omohyoid, and sternohyoid muscles) attach the

hyoid bone to inferior structures and act to depress the hyoid bone with assistance provided by elastic recoil of the trachea (Figure 1.4; van Lunteren & Strohl, 1988).

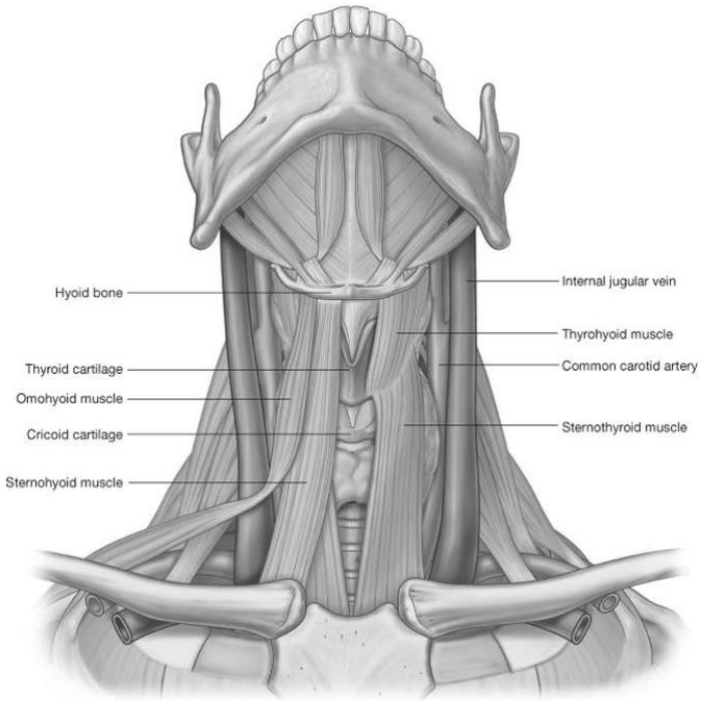

Figure 1.4. Anterior view of the infrahyoid 'strap' muscles. From Drake *et al.* (2010).

The four pairs of suprahyoid elevators (stylohyoid, digastric, mylohyoid, and geniohyoid muscles) pass in a superior direction from the hyoid bone to the skull or mandible (Figure 1.5). The intrinsic muscles of the larynx facilitate closing of the laryngeal inlet and control the movements of the vocal folds (van Lunteren & Strohl, 1988). The two oblique arytenoid muscles, which run from the posterior surface of one arytenoid cartilage to the apex of the arytenoid cartilage on the other side, are responsible for the former function with movement and control of the vocal folds undertaken by a further five muscles (posterior crico-arytenoid, transverse arytenoid, lateral crico-arytenoid, thyro-arytenoid, and the crycothyroid

muscles). All of the intrinsic laryngeal muscles are innervated by the recurrent laryngeal branch of the vagus nerve except for the crycothyroid muscle, which is supplied by the external branch of the superior laryngeal nerve. The anatomic location of the intrinsic muscles of the larynx is illustrated in Figure 1.6.

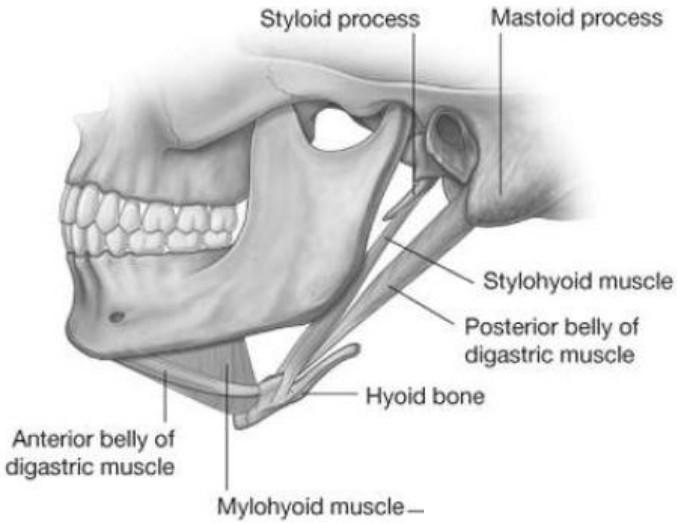

Figure 1.5. Lateral view of the suprahyoid elevator muscles. From Drake *et al.* (2010).

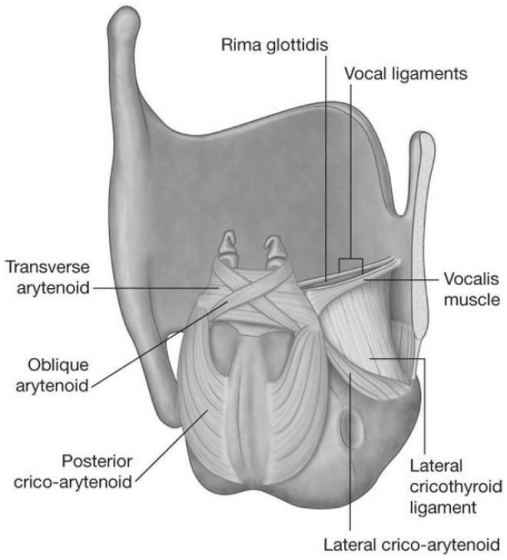

Figure 1.6. Anatomical arrangement of the intrinsic muscles of the larynx. From Drake *et al.* (2010).

All of the pharyngeal dilator muscles play a vital role in the maintenance of pharyngeal tone. The GG and GH are arguably the two most important. Both are major contributors to the patency of the airway during inspiration (Mathew *et al.*, 1982; Wiegand *et al.*, 1990) and demonstrate increased activation in response to pressure changes within the UA (Brouillette & Thach, 1979). Consequently, both are likely to demonstrate increased levels of activity during inspiratory loading.

1.2.2. Structure of the Lower Airway

The structures of the LA are primarily designed to provide a means of air distribution, humidification, and gas exchange between the atmosphere and pulmonary circulation. Functionally, the air passages can be divided into two zones; the conducting zone and the respiratory zone, with the 'regular dichotomy' model of Weibel and Gomez (1962) the most widely used description of the tracheobronchial tree. This model considers the airway as a series of numbered generations from the largest (trachea; generation 0) to the smallest (alveolar sac; generation 23). Each generation of bronchi branches successively into two smaller, equally sized bronchi, with the number of air passages within each generation being approximately 2 raised to the power of the specific generation number. Although a convenient model it does represent a rather simplistic approach that likely does not accurately follow the mathematical relationship described. In this respect, Sauret *et al.* (2002) used computed tomography (CT) to confirm that bifurcation only occurs as far as the sixth generation, after which some airways trifurcate, with others terminating as early as generation 8. The

following section will describe the structural characteristics of the major sub-divisions of the lower airway.

Trachea

The adult trachea (generation 0) is a tube-like structure that is approximately 11 cm long with a mean internal diameter of about 1.8 cm. The trachea extends from the larynx to the primary bronchi in the thoracic cavity. The walls of the trachea are comprised of U-shaped cartilaginous bands, which are joined on the posterior surface by bands of smooth muscle that act to reduce the size of the lumen and increase flow rate during expulsive manoeuvres such as coughing (Bartlett, 1989). The cartilage rings provide firmness to the trachea that helps to prevent collapse due to raised intrathoracic pressures associated with manoeuvres such as cough. The trachea is lined with pseudostratified ciliated columnar epithelium, a feature that is common throughout the respiratory tract.

Primary, lobar, and segmental bronchi

The primary, lobar, and segmental bronchi supply the individual lungs, lobes of each lung, and segments of each lobe, respectively (generations 1-4, Figure 1.7). The trachea divides at the lower end into primary bronchi with the right bronchus slightly larger and more vertical than the left. This anatomical detail explains why inspired foreign bodies tend to obstruct the right bronchus rather than the left. Structurally the primary, lobar, and segmental bronchi are similar to the trachea. U-shaped cartilage provides support in the primary bronchi with the cartilage rings becoming complete, but less regular in shape, in the lobar and segmental

bronchi. As for the trachea, ciliated mucosa lines this portion of the airway. These bronchi are sensitive to changes in intra-thoracic pressure that may limit expiratory flow when intra-thoracic pressure is raised above intra-luminal pressure, leading to collapse when the pressure difference exceeds 50 cmH_2O.

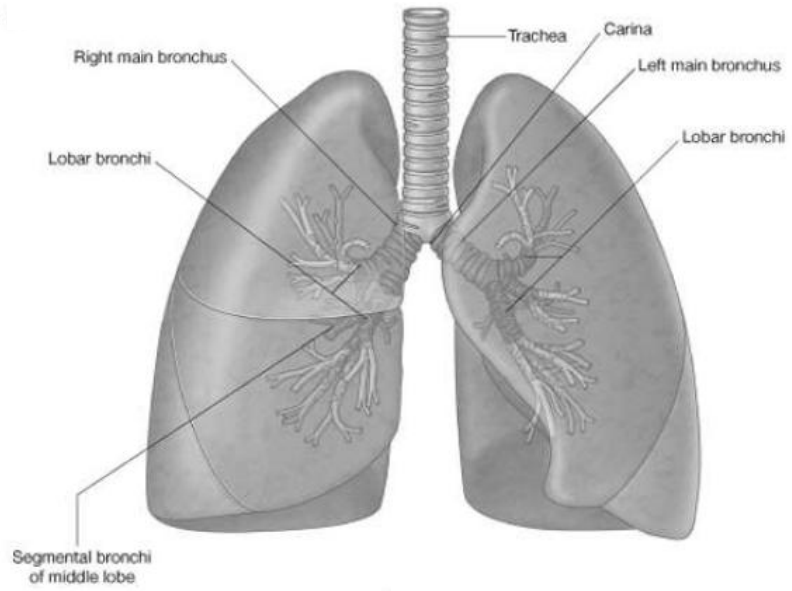

Figure 1.7. The tracheobronchial tree showing the primary, lobar and segmental bronchi. From Drake *et al.* (2010).

Small bronchi

The small bronchi branch from generations 5 to 11 with a gradual decrease in diameter from approximately 3.5 mm to 1 mm. These air passages rely on irregular plates of cartilage for structural support that are embedded in a coat of fibrous tissue. A layer of circular bronchial smooth muscle fibres is found internal to the fibrous coat and this itself is lined with a mucous membrane, lined by columnar ciliated epithelium. Due to their small size these air passages are also somewhat dependent on a positive transmural pressure gradient to 'assist' the cartilage with the maintenance of airway tone.

Bronchioles

The bronchioles extend from generations 12-14 and differ in structure from previous generations with the absence of cartilage to maintain airway tone. At this level the air passages are embedded within the parenchyma of the lung and it is the tethering forces of the parenchyma that prevent airway collapse. Consequently, the primary factor determining airway cross-section at this level of the bronchial tree, is lung volume. The tethering forces applied to the airway wall are greater at high lung volumes, with a consequent lowering of resistance to airflow, with lower lung volumes associated with an increase in airway resistance. The mean internal diameter of the bronchioles diminishes from 1 mm to 0.7 mm with each successive generation, along with a rapid increase in the total number of bronchioles. Consequently, the cross-sectional area of these airways is large, providing a low resistance to airflow under normal conditions, despite the diminishing size of the airway lumen. However, under conditions of ASM contraction, resistance to airflow can rise significantly. This will be discussed further in section 1.6.

Respiratory bronchioles

At airway generations 15-18 the functional role of the respiratory system moves toward one of gas exchange. Each bronchiole divides into two or more respiratory bronchioles that are distinct from bronchioles in that their walls are scattered with alveoli that increase in number with successive generations. Mean diameter is similar across all generations of respiratory bronchiole and approximates 0.4 mm.

Alveolar ducts

The amount of alveoli scattered along the wall of the terminal respiratory bronchioles increases to the point whereby it is solely the mouth of the alveoli that forms the 'wall' of the bronchiole (approximately 20 alveoli). When this occurs (generations 19-22) an alveolar duct is formed with approximately half the total amount of alveoli arising from these ducts.

Alveolar sacs and alveoli

The alveolar sac (generation 23) is the terminal portion of the alveolar duct and contains the other half of the total amount of alveoli. The zone supplied by a single terminal bronchiole, to include the respiratory bronchiole, alveolar ducts, and the alveolar sacs is known as the pulmonary acinus. There are approximately 30,000 acini, each between 5 and 12 mm in length and containing over 10,000 alveoli (Haefeli-Bleuer & Weibel, 1988). The total number of alveoli varies with lung size and ranges from approximately 270 to 790 million with a mean of 480 million (Ochs *et al.*, 2004).

The airways with an internal diameter of < 2 mm account for up to 35 and 60% of resistance to airflow in people with mild and moderate/severe asthma, respectively (Yanai *et al.*, 1992). Consequently, although some of the smaller bronchi are implicated, the largest contributor to airflow obstruction lies within the bronchioles. As discussed previously, lung volume is the primary determinant of airway cross sectional area at this airway generation level with two forces acting to dilate the airway as lung volume increases (An *et al.*, 2007). The first force

relates to the pressure difference acting across the airway wall, whilst the second relates to the tethering forces of the parenchyma (Lai-Fook *et al.*, 1978; Gunst *et al.*, 1988). It is likely that loaded breathing would act primarily to modulate the former force by increasing the transluminal pressure at volumes approaching TLC, beyond that normally achieved (~ 30 cmH_2O). Indeed, an increased transluminal pressure may provide an additional stimulus to induce relaxation of the stiffer ASM of people with asthma.

1.3. MECHANICAL FUNCTION OF THE UPPER AIRWAY DILATOR MUSCLES

As discussed in section 1.2.1 the functions of the UA include speech, deglutition, and breathing, with many of the muscles involved in deglutition reciprocally active in breathing. The dynamic process of breathing affects airway geometry due to the highly deformable nature of the oropharyngeal airway (Peslin *et al.*, 1984). The contraction of thoracic inspiratory muscles causes a decrease in pleural pressure that also results in a reduction in airway pressure below the atmospheric level. As a consequence the extra-thoracic airways experience a negative intraluminal pressure during inspiration that has a collapsing effect on the UA. The extent of the pressure change in the UA is determined by a number of factors that include the elasticity of the airway wall, the size of the force that is driving airflow, as well as how resistance is distributed throughout the UA (Olson *et al.*, 1988). With an absence of bony or cartilaginous structures to maintain UA patency at the pharyngeal level, UA patency is solely dependent on the activation of the UA dilator muscles to resist the collapsing effect of negative intraluminal pressure during inspiration (Series, 2002). In addition, activation of UA muscles also regulates the valve-like behaviour of the larynx by altering the shape and size of the laryngeal inlet and position of the vocal folds (Olson *et al.*, 1988).

This section will focus on the respiratory functions of the pharyngeal UA dilator muscles, GG and GH, as well as the role of the larynx in healthy and patient groups.

1.3.1. Upper Airway Function in Healthy Human Beings

The pharynx

The most studied muscles of the pharynx appear to be the GG and GH that act to prevent the tongue from falling into the pharyngeal lumen by gravity, or sub-atmospheric intra-pharyngeal pressure (Mitchinson & Yoffey, 1947). Activation of the GG protrudes and depresses the tongue whereas the GH elevates the hyoid bone and the base of the tongue (Figures 1.2 and 1.3). Motor control of the UA muscles is closely linked to the activity of the thoracic respiratory muscle motor neurones, with hypoglossal nerve activation paralleling that of the phrenic nerve, although with an earlier and near-maximal firing pattern (Strohl *et al.*, 1980; Hwang *et al.*, 1983). This is important as the integrity of the UA lumen during breathing requires rhythmic pre-inspiratory activation of the UA muscles to preserve the patency of the airway (Brouillette & Thach, 1979). The importance of the pre-inspiratory pattern of UA activation is particularly apparent in patients with respiratory insufficiency who are undergoing diaphragmatic pacing. Pacing of the diaphragm disrupts the temporal coordination between the UA muscles and diaphragm and leads to UA occlusion during sleep (Hyland *et al.*, 1981). In addition to the phasic inspiratory activity the GG also exhibits tonic activity during expiration (Akahoshi *et al.*, 2001; Saboisky *et al.*, 2006).

In addition to the centrally activated drive described above the GG also responds to locally mediated reflex mechanisms (Pillar *et al.*, 2001). Both tonic and phasic electromyogram (EMG) activity of the GG is know to increase as pressure within the UA decreases, with increases in pressure having the opposite effect (Mathew

et al., 1982). Further, Horner *et al.* (1991b) demonstrated that rapid changes in negative intra-pharyngeal pressure activates the GG with a median response latency of 34 ms. Voluntary activation of the GG was much slower (184 ms) indicating that activation due to sudden pressure change was likely due to a reflex response (Horner *et al.*, 1991b). A further study by the same group investigated the effect of the application of UA anaesthesia to desensitise the pharyngeal mechanoreceptors (Horner *et al.*, 1991a). Desensitising the UA resulted in a large decrease in the EMG response confirming that upper airway afferents are involved in mediating pharyngeal dilator muscle activation in response to negative pressure (Horner *et al.*, 1991a).The mechanical effect of UA muscle activation was demonstrated in the isolated airways of tracheotomised dogs. Fouke *et al.* (1986) showed a correspondence between force production of UA muscles and volume expansion in the upper airway, during active respiratory efforts. Inspiration was associated with a decrease in pressure, within the sealed UA, and an increase in volume (1 cm^3) when the airway was opened during resting ventilation. It has also been shown that UA pressure swings are augmented when central respiratory drive is increased by hyperoxic hypercapnia in dogs (Strohl & Fouke, 1985). Further, in humans increases in UA EMG activity due to hypoxia and hypercapnia are associated with reductions in pharyngeal resistance (Series *et al.*, 1989; Dinh *et al.*, 1991).

Evidence to support a neuromuscular mechanism for the maintenance of UA patency was presented by Brouillette & Thach (1979). These authors determined the critical pressure (P_{crit}) at which the UA collapses in the isolated UA of rabbits.

It was found that the UA still remained patent when exposed to negative pressures of -80 cmH_2O. Further, a more negative pressure was required to induce closure when EMG activity of the GG and GH was elevated, and when the hyoid bone was displaced anteriorly post mortem. After resection of the 12^{th} cranial nerve abolished EMG activity, airway closure occurred during inspiration, confirming that a neuromuscular mechanism was responsible for the maintenance of UA patency (Brouillette & Thach, 1979).

The mean inspiratory mouth pressures generated during IMT are typically -50 to -60 cmH_2O. Pressures such as these would be sufficiently negative to induce a reflex activation of the GG and GH concomitant to a centrally activated pre-inspiratory activation. Further, whilst the negative pressure associated with IMT are sufficient to augment GG activation they are unlikely to induce airway collapse (Brouillette & Thach, 1979). The prolonged and repeated nature of the inspirations associated with IMT may also provide sufficient stimulus to the GG and GH to induce a training response.

The larynx

In contrast to the pharynx, the larynx is protected from inspiratory related collapse due to the effect of the cricoid cartilage that forms a relatively rigid ring surrounding the laryngeal airway (Bartlett, 1989). The vocal folds provide a valve-like mechanism that regulates pressure across the glottis, resistance to airflow, and in certain circumstances, helps to regulated end-expiratory lung volume (Brancatisano & Engel, 1988). At rest the vocal folds exhibit phasic

activity that is closely coordinated with the diaphragm and inspiratory muscles of the chest wall (England *et al.*, 1982). Phasic activation of the posterior crico-arytenoid (PCA) muscle (Figure 1.6) abducts the vocal folds, in advance of inspiratory flow, where they reach their widest point at mid-inspiration (Brancatisano & Engel, 1988). PCA activity is lower during the expiratory phase, permitting passive recoil of the vocal folds back toward the midline, slowing airflow, and raising laryngeal resistance, until approximately two-thirds of the tidal volume is expired (England *et al.*, 1982; Bartlett, 1989).

The reciprocal relationship between diaphragm and PCA activation is similar to that of other UA muscles (Brancatisano *et al.*, 1984). This relationship may be exploited during IMT to increase the inspiratory activation of the PCA concomitant with that of the diaphragm. It has previously been proposed that there may be a synergistic training effect within the diaphragm and PCA with IMT, which may result in increased glottal aperture size and reduced laryngeal resistance (Ruddy *et al.*, 2004). The potential longer-term effect of IMT on the PCA is discussed in section 1.4.

Effect of exercise on upper airway function

The elevated ventilation associated with exercise presents an additional challenge to the integrity of the UA. Minimisation of UA resistance may be mediated by increased neural drive to the GG that would contribute to a stiffening and/or dilation of the UA. To investigate the effect of increases in ventilation on neural drive to the UA Williams *et al.* (2000) compared the EMG activity of the GG and

nasal dilator muscles (NDM) during incremental exercise in the upright and supine postures. They reported a near linear increase in GG and NDM EMG activity with exercise intensity. In addition, EMG activity of the GG reached ~ 40 % of maximal values at exercise termination; a value approaching levels of drive seen in the diaphragm during maximal exercise (Johnson *et al.*, 1993). Activity of the GG was not affected by posture although GG EMG activity did increase at higher exercise intensities when breathing switched from oro-nasal to a predominantly oral route (Williams *et al.*, 2000). Stability of the UA has also been shown to increase during hypercapnia-induced hyperventilation in dogs; the critical pressure at which the UA collapses was raised during hyperventilation from -4.3 to -8.5 cmH_2O, suggesting improved stability and patency of the UA (Oliven *et al.*, 1989). In addition, UA resistance decreased linearly as PCO_2 and ventilation increased over the course of CO_2 re-breathing, suggesting that increased UA muscle activity improves UA patency by decreasing resistance to airflow and increasing UA wall rigidity and stability against collapse (Oliven *et al.*, 1989).

During conditions of hyperpnoea, such as those associated with exercise, inspiratory abduction of the vocal folds is wider, reflecting increased neural drive to the PCA muscles (England & Bartlett, 1982; Bartlett, 1989). Under similar exercise conditions, studies in animals have measured small reductions in laryngeal resistance, which support observations made in humans (McCaffrey & Kern, 1980). The consequence of a wider glottic aperture and reduction in laryngeal resistance would be a reduction in the driving pressure requirement for a

given inspiratory flow rate (Brancatisano & Engel, 1988). A continuation of the inspiratory abduction is seen throughout the expiratory phase with a concomitant reduction in laryngeal resistance (Spann & Hyatt, 1971; England & Bartlett, 1982).

Post-IMT improvements in UA function may lead to a lower resistance to airflow and an associated reduction in the work of breathing. Furthermore, reductions in motor output associated with increased strength of the UA muscles following IMT may contribute to a reduced perception of breathing effort during exercise (Spengler, 2002; Ruddy *et al.*, 2004). It is well accepted that respiratory effort perception is reduced post- IMT (Volianitis *et al.*, 2001; Romer *et al.*, 2002a) and the reduction in effort perception is usually attributed to a reduced motor command to the thoracic respiratory muscles (McConnell & Romer, 2004). An additional factor worthy of consideration may be related to potential improvements in the function of pharyngeal and laryngeal dilator muscles following IMT.

Strength and endurance characteristics of the genioglossus

The strength and endurance characteristics of the GG have been reported in young men and women, as well as older people (Scardella *et al.*, 1993; Mortimore *et al.*, 1999). Gender differences in tongue protrusion force are negated after normalising for fat free mass, although age related reductions in maximal force have been shown (Mortimore *et al.*, 1999). In common with other skeletal muscle, Scardella *et al.* (1993) reported a progressive reduction in time to the limit of tolerance

during repeated tongue protrusions when higher percentages of maximal force (80% maximal voluntary contraction; (MVC)) were required. In addition, it was reported that 10 min of hypercapnic inspiratory resistive loading at 80% of GG MVC was insufficient to induce fatigue in the thoracic inspiratory muscles, but was sufficient to reduce the endurance capacity of the GG.

1.3.2. Upper Airway Function in Pathological States

Obstructive sleep apnoea syndrome

Obstructive sleep apnoea syndrome (OSAS) is characterised by narrowing of the upper airway during sleep that leads to airway obstruction, arterial oxygen de-saturation, and occasionally a rise in arterial CO_2 content (Verbraecken & De Backer, 2009). Ongoing breathing effort continues throughout the duration of the obstructive episode and is associated with surges in sympathetic neural activation (Eckert & Malhotra, 2008). Loud snoring often accompanies OSAS with cessation of the apnoeic episode often due to awakening from sleep. The accepted method of assessing the severity of OSAS is determined by polysomnography, which provides an objective measure known as the apnoea-hypopnoea index (AHI). Diagnosis of OSAS is confirmed when the AHI is over 5 and using this criterion the prevalence of OSAS is thought to be between 3.5 and 24% in men and 1.5 and 9% in women (Strohl & Redline, 1996; Ohayon *et al.*, 1997). In severe cases the patient may suffer in excess of 100 events per hour, each lasting approximately 30 s (Eckert & Malhotra, 2008).

Several factors can explain airway occlusion during sleep, including abnormal UA anatomy, with an increased propensity for UA collapse, as well as impaired UA dilator muscle activation (Gleadhill *et al.*, 1991; Mezzanotte *et al.*, 1992; Schwab *et al.*, 1995). These factors are discussed below.

It is widely accepted that patients with OSAS have airways that are anatomically narrower compared to healthy individuals; although there is some degree of overlap (Schwab *et al.*, 1995). One reason for the narrower airway is related to obesity and the strong relationship between OSAS and neck circumference supports this assertion (Davies & Stradling, 1990). In obesity there is an accumulation of peri-pharyngeal fat that is associated with lateral narrowing of the airway (Mortimore *et al.*, 1998). In OSAS fat accumulation in the UA affects airway geometry whereby the configuration of the major axis is anterior-posterior, compared to a lateral configuration in healthy individuals (Schwab *et al.*, 1993; Schwab *et al.*, 1995). Generally, a narrower airway is more susceptible to collapse making the UA of the OSAS patient vulnerable from an anatomic perspective (Eckert & Malhotra, 2008). The collapsibility of the airway also plays an important part in OSAS with higher levels of collapsibility associated with increased levels of airflow obstruction (Gleadhill *et al.*, 1991). It is the compliance of the walls of the laryngopharynx that determine the ease with which the UA collapses due to its particular susceptibility (O'Donnell *et al.*, 2000). Upper airway collapsibility was assessed by Isono *et al.* (1997) who found that the P_{crit} in OSAS patients was significantly higher (less negative) than controls, confirming that the UA of OSAS patients is more collapsible largely due to anatomic compromise.

The P_{crit} is largely determined by airway anatomy, although stimulation of the hypoglossal nerve during inspiration (increasing airway cross-sectional area), has been shown to lower the $P_{crit,}$ as well as improving the AHI (Oliven *et al.*, 2003).

Despite narrower airways, muscular stabilisation of the airway in OSAS ensures that airway area remains constant during inspiration, as in healthy individuals. The implication of this is that the UA dilator muscles are able to prevent airway collapse associated with negative intra-luminal pressure during inspiration. However, during expiration, and after an initial increase in airway calibre due to positive intra-luminal pressure, there is a subsequent narrowing of the airway at end-expiration that is significantly more pronounced in patients with OSAS (Schwab *et al.*, 1995). It would, therefore, appear that end expiration is the point in the respiratory cycle when lateral narrowing of the pharyngeal walls in OSAS patients becomes critical (Verbraecken & De Backer, 2009).

When awake, patients with OSAS have a higher degree of GG UA dilator activation compared to controls, which is thought to be a physiological response to a narrower airway (Mezzanotte *et al.*, 1992; Fogel *et al.*, 2001). However, during sleep the negative pressure reflex pathway to many UA dilators is less active than during wakefulness (Remmers, 2001). Further, muscles of the nasopharynx, which exhibit a predominantly tonic activity pattern, show a gradual reduction in activity as sleep progresses, leading to an increase in resistance within the UA (Tangel *et al.*, 1991). Phasic inspiratory activity of the GG and GH is maintained throughout sleep although the magnitude of that activity is reduced

further than that seen in healthy people (Mezzanotte *et al.*, 1996; Henke, 1998). In effect, the higher level of UA dilator activation required to maintain airway patency during wakefulness is diminished during sleep. When this is coupled with increased airway resistance due to reduced drive to tonic UA dilators, and a loss of reflex activation of UA dilators, the likelihood of airway collapse is increased. The maintenance of pharyngeal patency during sleep has been described by the 'balance of forces' model that reflects both the mechanical loads that tend toward airway collapse and the reflex activation of UA dilators that is suppressed during sleep (Figure 1.8; Brouillette & Thach, 1979; Verbraecken & De Backer, 2009).

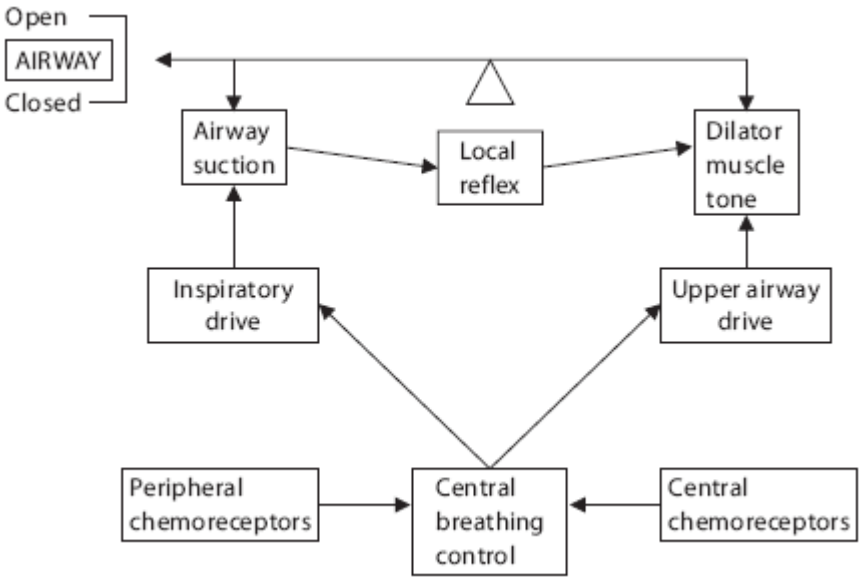

Figure 1.8. The balance of forces model of upper airway collapse. From Verbraecken & De Backer (2009).

Recently, Shepherd *et al.* (2006) described a linear relationship (r = 0.61; P < 0.001) between tongue protrusion force and maximal inspiratory mouth pressure (MIP) in patients with OSAS. Although no association was seen between AHI and MIP or tongue protrusion force it was reported that moderate-to-severe OSAS was

not seen in individuals with a ratio of maximum tongue protrusion force to MIP that was greater than the 90th percentile. Although a strong relationship between the ratio of maximum tongue protrusion force to MIP and severity of OSAS did not exist, the authors suggested that a ratio that exceeded the 90th percentile may be protective against UA collapse (Shepherd *et al.*, 2006).

The evidence presented above supports the rationale for targeting the development of UA dilator muscle strength as a means of improving UA function (Steele, 2009). The absence of a relationship between AHI and either MIP or tongue protrusion force suggests that the linear relationship between tongue protrusion force and MIP is likely an inherent characteristic rather than one caused by OSAS (Shepherd *et al.*, 2006). Consequently, IMT may be a method that can be used to improve the ratio of UA muscle strength to MIP. However, careful consideration to the choice of optimum load would be required to ensure the load was sufficient to induce a training response in the UA, whilst providing a minimal stimulus to the thoracic inspiratory muscles.

Vocal fold dysfunction

Movement of the vocal folds during quiet breathing acts as a choke that provides fine control of airway resistance (Gal, 1990). On inspiration, phasic activation of the PCA muscle abducts the vocal folds and acts to minimise resistance to airflow (Brancatisano *et al.*, 1984). During expiration the thyro-arytenoid muscle adducts the vocal folds with a concomitant increase in airway resistance (Kuna *et al.*, 1988). The fine control of airway resistance during expiration has been suggested

to be important in determining expiratory time and, perhaps, in the prevention of lower airway collapse (England *et al.*, 1982; Gal, 1990). During exercise, ventilatory frequency increases, predominantly by a reduction in expiratory time. The hyperpnoea of exercise is associated with less activity of the thyro-arytenoid muscle and consequently less adduction of the vocal folds and a reduction in resistance to expiratory airflow (England & Bartlett, 1982). Exercise is also associated with increased abduction of the vocal folds during inspiration, which also lowers resistance to airflow (England & Bartlett, 1982).

The most common presentation of exercise associated UA obstruction is a condition known as paradoxical vocal fold motion (PVFM). PVFM is specifically characterised by the adduction (rather than abduction) of the vocal folds during inspiration (McFadden & Zawadski, 1996). Symptoms associated with PVFM include dyspnoea, inspiratory wheeze (stridor), and increased inspiratory effort, that are most likely to be only apparent during exercise (Weiss & Rundell, 2009). Consequently, definitive diagnosis using fibreoptic rhinolaryngoscopy is difficult, with symptom-based diagnosis more common. Indicative symptoms include a history of inspiratory wheeze and throat tightness that resolves shortly after cessation of exercise (Dickinson *et al.*, 2007). In addition, spirometry may provide evidence to support a diagnosis with a blunting of the inspiratory portion of the maximal flow-volume loop and a mid flow ratio (FEF_{50}/FIF_{50}) > 1.5 (Rundell & Spiering, 2003; Mathers-Schmidt & Brilla, 2005). The prevalence of PVFM is unclear although it is thought to be 5 – 15% in subjects experiencing inappropriate dyspnoea during exercise and appears to be more common in females (Rundell &

Spiering, 2003; Abu-Hasan *et al.*, 2005). More importantly, the specific pathophysiology of PVFM is unknown with UA irritation, laryngeal dystonia, and psychogenic aetiologies all suggested as potential causes (Ruddy *et al.*, 2004).

1.4. PLASTICITY OF THE UPPER AIRWAY

The muscles of the UA are skeletal and should, therefore, respond to overload in the same way as other skeletal muscle. Chronic overload of skeletal muscle is associated with a transformation in the muscle morphological characteristics that reflects the nature of the stimulus (Harridge, 2007). The normal adult human skeletal muscle fibre expresses three myosin heavy chain (MHC) isoforms: the slow MHC-I, and the two fast, MHC-IIa and MHC-IIx (Harridge, 2007). Chronic endurance and resistance training are known to result in a transformation from MCH-IIx to MCH-IIa isoforms, with inactivity and immobilisation associated with a shift in the opposite direction (Andersen & Aagaard, 2000). In addition, intense sprint training is associated with a MCH-I to MCH-IIa transformation, with the reverse reported after endurance training (Baumann *et al.*, 1987; Allemeier *et al.*, 1994).

The intrinsic and extrinsic muscles of the tongue express a significantly greater proportion of MHC-IIa and MHC-IIx fibre types (Brozanski *et al.*, 1993; Polla *et al.*, 2004) that reflects the wide variety of functions undertaken by the muscles of the UA. For example, in addition to its role as an UA dilator muscle, the GH is required to generate high forces and fast contractile speeds during the act of swallowing, which require a greater proportion of MCH-II isoforms (Smith *et al.*, 2005). The inherent characteristics of the UA muscles reflect their function and consequently, when functional requirements change, the UA muscles should adapt accordingly. Evidence exists to support both adaptive and maladaptive changes in

the UA muscles depending on the stimulus (Kimoff, 2007). These factors will be discussed below.

Studies involving human participants have shown that GG muscle activity increases as a function of exercise intensity (Williams *et al.*, 2000). Evidence to support an adaptive response to exercise is absent in healthy human beings, but positive training adaptations are known to occur in rodents. For example, Vincent *et al*. (2002) reported an increase in the expression of MHC-I and a decrease in MHC-IIb (synonymous with the MCH-IIx in humans) phenotype of the UA muscles (digastric and sternohyoid) in response to exercise hyperpnoea in rodents. In addition, an increase in the oxidative biochemical characteristics of the UA muscles was also reported. Although no changes were seen in the GG, the authors acknowledge that this may be related to a lack of any sustained increase in GG EMG seen during exercise (Vincent *et al.*, 2002). The lack of GG EMG response in rodents, compared to human beings, may also have been due to between species differences or, perhaps, an insufficient training stimulus. Either way, it seems reasonable to suggest that the known association between GG activity and exercise intensity in human beings may result in structural adaptations similar to those seen in rodents, should the training stimulus be of sufficient magnitude.

Additional evidence that supports the notion that the UA responds to an overload stimulus is provided by studies that have described the UA muscles of patients with OSAS. Patients with OSAS exhibit augmented activity of the GG and other UA muscles during wakefulness that is thought to represent a compensatory

mechanism to preserve UA patency (Mezzanotte *et al.*, 1992). Specifically, the characteristics of the soft palate muscle, musculus uvulae (MU), shows a greater capacity to generate tension in OSAS patients compared to snorers (Series *et al.*, 1995). Further, GG biopsies from patients with OSAS have been shown to exhibit greater fatigability than those taken from control subjects (Carrera *et al.*, 1999). In addition, evidence of muscle injury, inflammation, and an increase in MHC-IIa content have been reported in UA dilator muscles in OSAS patients (Petrof *et al.*, 1994; Series *et al.*, 1995; Carrera *et al.*, 1999). Series *et al.* (1996) reported a higher proportion of MHC-IIa content in the GG that was associated with a decrease in the content of MHC-IIx. The down-regulation of MHC-IIx isoforms in response to fast, phasic patterns of activity is well reported and it seems likely that the morphological changes seen in OSAS patients represent a maladaptive response to the augmented GG activity seen during wakefulness (Kimoff, 2007).

Indirect evidence of improvements in UA function following UA training has been provided by a number of studies. Furrer *et al.* (1998) used four weeks of voluntary isocapnic hyperpnoea to train the UA dilator muscles in people who snored, but were otherwise healthy. There was a significant reduction in the incidence of snoring, as well as a significant increase in the volume of the GG, suggesting a causal link between the chronic activation of upper airway dilators and improvements in symptoms. In a novel study, Puhan *et al.* (2006) investigated the effect of 4 months didgeridoo playing (6 $d \cdot wk^{-1}$; 25 $min \cdot d^{-1}$) on various outcomes of sleep quality. The involvement of UA muscles in didgeridoo playing has previously been described (Tarnopolsky *et al.*, 2005). Compared to control,

significant improvements in AHI (an objective measurement of OSAS severity) and daytime sleepiness were reported, which the authors attributed to a reduced collapsibility of the upper airways after training (Puhan *et al.*, 2006).

The improvements in functional outcomes reported above suggest that the muscles of the UA are activated by breathing related exercises. However, without knowing the extent of activation of specific UA muscles it is difficult to establish a causative link between UA training and the functional outcomes reported in these studies. In an attempt to address this, Randerath *et al.* (2004) used a double-blind, placebo controlled design to specifically train the GG of patients with OSAS. Activation of GG by electrical neurostimulation (20 min, twice a day, for 8 weeks) was shown to reduce the incidence of snoring, but not the incidence of apnoea, in 33 OSAS patients compared to a placebo group (Randerath *et al.*, 2004). Unfortunately, a weakness of this study is the absence of morphologic or anatomical measurements that would support the observational findings. More recently, Guimaraes *et al.* (2009) examined the effect of oropharyngeal exercise in patients with OSAS. Participants performed a range of oropharyngeal exercises that are commonly used to treat speech pathology and are designed to recruit a range of UA muscles. Compared to control, 3 months of oropharyngeal exercise was associated with a 39% reduction in the AHI. Further, significant improvements were also seen in snoring, daytime sleepiness, and sleep quality (Guimaraes *et al.*, 2009).

The evidence presented above supports the notion that the muscles of the pharyngeal UA respond to training stimuli in a way that is similar to other skeletal muscles. Further, there is compelling evidence to suggest that novel training exercises of UA muscles may lead to some improvement in outcomes of sleep-disordered breathing. Neural drive to the UA muscles is reduced during sleep leading to an imbalance of airway pressure and dilating force (Brouillette & Thach, 1979). The augmented neural drive to the UA muscles of OSAS patients, whilst awake, means that the loss of drive whilst asleep, results in a greater imbalance of airway pressure and dilating force compared to healthy people (Mezzanotte *et al.*, 1996). Consequently, any training effect carried over to the sleep state may be due to improvements in the passive tone of the UA muscles and an associated increase in pharyngeal wall stiffness (Lindstedt *et al.*, 2002).

Laryngeal airway

A number of case studies have documented successful outcomes after the prescription of IMT to alleviate the symptoms of PVFM. These reports have involved individuals with both elite and non-elite athletic backgrounds (Ruddy *et al.*, 2004; Mathers-Schmidt & Brilla, 2005; Dickinson *et al.*, 2007) as well as non-athletic adults and children (Baker *et al.*, 2003a; Baker *et al.*, 2003b). Patients typically present with unexplained dyspnoea and inspiratory stridor and are prescribed a training period of pressure-threshold IMT over a number of weeks. For example, Dickinson *et al*. (2007) described the support provided to an elite female swimmer who reported dyspnoea and wheezing during high intensity training. After an 11-week period of twice daily IMT at 50-60% MIP the athlete

reported an absence of symptoms during the high intensity exercise that previously triggered the symptoms.

The mechanisms by which IMT may improve symptoms of PVFM are unclear. It has been suggested that increasing the strength of the thoracic inspiratory muscles reduces the need to adopt maladaptive excessive laryngeal tension at the level of activity that elicits the response (Mathers-Schmidt & Brilla, 2005). Furthermore, greater inspiratory muscle strength would also make overcoming the raised level of resistance associated with UA obstruction easier. Another mechanism may be related to the consistent finding that IMT is associated with a lowered perception of dyspnoea during exercise (McConnell & Romer, 2004). A lowered perception of dyspnoea may help to mitigate any potential psychogenic aetiology for PVFM by reducing the perceived panic and struggle behaviour seen in some individuals (Mathers-Schmidt & Brilla, 2005). A more direct mechanism has been proposed that suggests that IMT may take advantage of the reciprocal relationship between diaphragm and PCA innervation (Baker *et al.*, 2003a). It is known that PCA and diaphragm activity are in phase, with PCA activity occurring prior to the onset of inspiration (Brancatisano *et al.*, 1984). In addition, it has also been demonstrated that activity of this muscle is higher with sustained glottic widening, during respiratory manoeuvres such as panting (Brancatisano *et al.*, 1984). Furthermore, a coordinated increase in PCA activity is seen during phrenic nerve stimulation of the diaphragm (van Lunteren *et al.*, 1983). Consequently, it is plausible that the enhanced activity of the diaphragm required to overcome a threshold inspiratory

load would also increase the activity of the PCA to a level that may result in a training effect that enhances the function of this UA dilator muscle.

1.5. ASSESSMENT OF UPPER AIRWAY FUNCTION

A number of techniques are available for the assessment of UA muscle activation and two of the most commonly used methods are EMG and magnetic resonance imaging (MRI). In addition to assessing muscle activation, MRI has the additional benefit of enabling the measurement of soft tissue structures.

1.5.1. Electromyography

EMG is an experimental technique that allows the recording and analysis of myoelectric signals, or action potentials, as they propagate along the sarcolemma of muscle fibres from the neuromuscular junction to the ends of the fibres. The myoelectric signals can be recorded using either surface or intra-muscular electrodes. The use of intra-muscular EMG has been used by a number of researchers investigating the GG (Eastwood *et al.*, 2003; Saboisky *et al.*, 2006), however, its use is best suited to low intensity, short duration, muscle activation due to the susceptibility of the electrodes to movement (O'Connor *et al.*, 2007). Further, the invasive nature of the technique makes it unsuitable for use in many applications that rely on the use of human volunteers.

In contrast, surface electrodes provide a non-invasive technique that is suitable for recording the activity of the muscle as a whole (O'Connor *et al.*, 2007). Also, the surface EMG provides increased stability of electrode placement that is important when measurements are recorded over a long duration or when between day comparisons are needed. However, due to the location of the primary UA dilator muscles, the use of surface EMG requires bespoke equipment that is generally

only suited to investigating the activity of the GG and not other important UA muscles (Doble *et al.*, 1985; O'Connor *et al.*, 2007).

In view of the limitations of EMG in respect of the aims of this thesis, MRI provides an attractive alternative to quantify both UA muscle activation and UA dimension.

1.5.2. Magnetic Resonance Imaging

MRI provides a safe non-invasive method to quantify upper airway size and anatomy (Rodenstein *et al.*, 1990). In addition, post exercise MRI can also be used to provide information as to the extent of skeletal muscle recruitment during physical activity (Fleckenstein *et al.*, 1988). The absence of known risks relating to exposure to magnetic fields up to 3.0 tesla (T) has facilitated the progress of studies using healthy volunteers (Patten *et al.*, 2003). MRI uses radio frequency signals for image acquisition, and as such, the ethical concerns associated with imaging techniques that utilise ionising radiation, do not apply.

Exposure to the magnetic field of an MRI scanner causes hydrogen nuclei in tissue water to align. Perturbing the alignment of these elements, with magnetic pulses delivered at resonant frequency, causes the hydrogen nuclei to oscillate synchronously (phase-coherent oscillation) generating a detectable signal that can be recorded and manipulated to create an image. On cessation of the magnetic pulse, the phase-coherence begins to break down and the signal begins to decay. The rate at which the nuclei de-phase and realign (relaxation rate), after the

magnetic pulse ceases, is known as the transverse relaxation time or T_2. The image signal intensity is dependent on two properties of the tissue being imaged; 1) the concentration of hydrogen nuclei within the tissue being imaged, and 2) the T_2 of the hydrogen nuclei within the tissue environment. For example, bone shows up as black on MR images as it contains few hydrogen nuclei and the relaxation rate is fast, whereas fat, which contains higher concentrations of hydrogen, shows up much brighter in images due to its slower relaxation rate and higher concentration of hydrogen molecules (Meyer & Prior, 2000). The T_2 reflects the time constant of the signal decay and it is the increase of this time constant, associated with muscle metabolic activity, that is revealed by increased signal intensity in MR images (Meyer & Prior, 2000). Practically speaking, post-exercise images are brighter in comparison to pre-exercise.

Exercise-induced enhancements in the contrast of MR images was first reported by Fleckenstein *et al.* (1988). These authors reported changes in signal intensity that allowed active and inactive muscle to be distinguished from each other. Further, these authors also identified a moderate correlation ($r = 0.63$) between the level of exertion and signal intensity. Although the authors speculated that limitations in the measurement of work may have masked a stronger association. Additional studies by the same group have reported a strong proportional relationship between T_2 and the level of exertion (Fleckenstein *et al.*, 1989) and others have shown the relationship to be linear (Fisher *et al.*, 1990; Yue *et al.*, 1994).

The precise mechanisms behind post exercise increases in the T_2 of muscle water are still debated, but appear to be related to exercise induced increases in muscle water content (Meyer & Prior, 2000). Specifically, fluid shifts into the muscle cell, driven by activity related osmolites such as lactate (H^+) and phosphate ions, dilute the effect muscle proteins have on the T_2 of water (Patten *et al.*, 2003). In support of this hypothesis is the observation that McArdle's disease patients who lack phosphorylase, the enzyme required to metabolise glycogen, do not exhibit metabolite related increases in T_2 (Fleckenstein *et al.*, 1991). In addition, Prior *et al.* (2001) showed that increases in T_2 are more pronounced in muscle with fast twitch characteristics and high levels of ATPase activity. Further, it has been known for some time that larger increases in T_2 are seen in concentric compared to eccentric muscle activations (Shellock *et al.*, 1991), which is in line with the level of metabolic activity associated with these forms of activation.

Irrespective of the precise mechanism responsible for post exercise increases in T_2, there is a large body of information that supports its use for imaging activation patterns in various muscle groups. A strong association between EMG activity and T_2 of the biceps brachii was identified by Adams *et al*. (1992). These authors proposed that the use of T_2 as an index of muscle use was supported by the strong correlation ($r = 0.99$, $P < 0.05$) between these two independent measures. Fisher *et al.* (1990) proposed that the extent of the post exercise increase in T_2 was related to the amount of force produced during exercise. Over three consecutive bouts of graded ankle dorsiflexion it was found that T_2 was higher the greater the mean force produced during the activity. This association between T_2 and the

amount of work performed by the muscle was supported and extended by Yue *et al*. (1994). These authors also described a linear relationship between T_2 and work performed but only within a finite range. Increases in T_2 were smaller when work was increased from an already high value, which is in agreement with Fleckenstein *et al*. (1993).

A number of studies have used MRI to assess the UA in healthy individuals and OSAS patients, confirming its suitability for quantifying UA dimensions (Schwab *et al.*, 1995; Stuck *et al.*, 2002). The within-subject, between day, coefficient of variation for various UA dimensions measured at rest are reported to be between 3 and 19% (Stuck *et al.*, 2002). The most stable measurements are those of soft tissue structures, such as the tongue, and distances taken from a fixed base (e.g. the mandible). Airway spaces appear to have a higher degree of variability. Given the degree of variability in UA structures and spaces, careful selection of parameters is necessary to assess any changes due to an intervention. It is possible, however, that measurements taken during a loaded inspiration may become more stable than those taken at rest, due to an increase in the active tone of the UA.

Other studies have reported the T_2 relaxation characteristics of the GG and GH (Schotland *et al.*, 1999). Schotland *et al*. (1999) reported chronically increased T_2 values in the GG and GH of patients with OSAS compared with asymptomatic controls. The authors concluded that the altered properties of the UA muscles were compatible with muscle overuse syndrome.

In view of the above and in the context of this thesis it is known that the GG and GH are important UA dilator muscles that can be suitably identified on MR images (Schotland *et al.*, 1999). It is also known that changes in the T_2 value of these muscles, due to muscle overuse, can be identified. If IMT provided a sufficient exercise stimulus to the GG and GH it is, therefore, reasonable to assume that evidence for any increased activation would be identifiable by increases in the T_2 relaxation rate.

In summary, MRI provides a safe, non-invasive technique for quantifying the structure of the UA. In addition, enhancements in the contrast of MR images, as seen by increases in T_2, provide an objective method of analysing intact airway muscles. Further, the non-invasive nature of MRI allows repeated measurements to be carried out in the same participants.

1.6. MECHANICAL FUNCTION OF THE LOWER AIRWAY SMOOTH MUSCLE

The rate of flow through the bronchial airways is determined by the pressure gradient from the mouth to alveoli and the resistance to airflow provided by the respiratory tract. Under conditions of laminar flow, resistance is proportional to the viscosity of the gas (air) and the length of the airway, and has a negative relationship to the fourth power of the radius of the airway. This relationship is described by Poiseuille's Law for laminar flow (Equation 1).

Equation 1: $$\text{Flow rate} = \frac{\Delta P \times \pi \times (\text{radius})^4}{8 \times \text{length} \times \text{viscocity}}$$

ΔP = pressure gradient

This equation can be rearranged to give resistance (Equation 2).

Equation 2: $$\text{Resistance} = \frac{8 \times \text{length} \times \text{viscocity}}{\pi \times (\text{radius})^4}$$

Consequently, as gas viscosity and tube length remain fairly constant in the airway the major determinant of resistance to airflow is the radius of the airway. The functional significance of this is that for a given airway pressure, airway resistance will increase by 26% for every 5% reduction in airway radius, with a consequent reduction in flow rate (Gotshall, 2006). Further, the movement of air

along the pulmonary airways is actually a combination of laminar and turbulent flow, with turbulent flow exhibiting greater sensitivity to changes in airway radius.

1.6.1. Mechanical Factors Determining Lower Airway Respiratory Resistance

In the healthy lung, a change in the diameter of the small airways and bronchioles is the main factor controlling airway resistance (R_{aw}). A change in the diameter of the airway can occur via changes in lung volume or by contraction of ASM. It has been known for some time that lung volume is a key determinant of R_{aw}, with higher lung volumes associated with lower R_{aw} in both healthy people and patients with asthma (Briscoe & Dubois, 1958; Macklem & Mead, 1967; Jensen *et al.*, 2001; Torchio *et al.*, 2006). Consequently, R_{aw} is inversely related to lung volume when variables such as ASM tone remain stable (Lumb, 2005). Alongside changes in lung volume the calibre of the airways are also dependent on the tonic state of the ASM as well as the transmural pressure gradient (Jensen *et al.*, 2001). A direct association between ASM contractility and changes in lung volume is yet to be shown (An *et al.*, 2007), but it is likely that the mechanism underlying the influence of lung volume upon R_{aw} is twofold. Firstly, a modulation of the ASM length due to parenchyma pull; secondly, the pressure difference acting directly across the airway wall (Jensen *et al.*, 2001; Seow & Fredberg, 2001).

1.6.2. Neural Control of Airway Diameter

In the healthy lung, the primary mechanism regulating ASM tone is parasympathetic-cholinergic neural control via the vagus nerve (Canning & Fischer, 2001). A degree of vagal tone is generally present at rest and withdrawal of parasympathetic activity, such as seen during exercise, acts to relax the ASM, resulting in a degree of bronchodilation (Pichon *et al.*, 2005). When activated fully, the parasympathetic nerves innervating the airways are capable of inducing complete closure of the smaller bronchi and bronchioles, as well as substantially increasing resistance to airflow in the larger airways (Canning & Undem, 1993). Thus, the degree of readily reversible baseline tone in the airway likely reflects a balance between the activation of contractile and relaxant parasympathetic nerves (Canning & Fischer, 2001).

The sympathetic nervous system is poorly represented in the lung and consequently direct sympathetic innervation is not considered to play a significant role in bronchial smooth muscle regulation (Lumb, 2005). However, despite the limited role for sympathetic innervation, bronchial smooth muscle does have an abundance of β_2- adrenergic receptors. Stimulation of β_2- adrenergic receptors by circulating adrenaline may act directly to reduce smooth muscle tone, or indirectly by inhibiting acetylcholine release from cholinergic nerve fibres (Thomson *et al.*, 1996). Although levels of circulating adrenaline probably are not important in regulating smooth muscle tone at rest, this mechanism is likely more important during exercise (Warren & Dalton, 1983). The β_2- adrenergic receptor has

particular relevance in asthma as it provides the target site for β_2-agonist drugs that act to relax ASM.

Evidence exists for a third autonomic nervous control system that neither uses adrenaline nor acetylcholine as its neurotransmitter and may act as the sole bronchodilatory nervous pathway (Fischer & Hoffmann, 1996). Efferent fibres from this non-adrenergic, non-cholinergic (NANC) system act directly on ASM causing relaxation. The process is likely mediated by release of the neurotransmitter vasoactive intestinal peptide (VIP), with subsequent production of nitric oxide (NO) interacting with the ASM cell to promote relaxation (Canning & Fischer, 2001; Lumb, 2005). Although airway calibre at rest is modulated by NO it is, unclear whether this is via innervation of NANC fibres or by local release of NO by the ASM cell (Canning & Fischer, 2001).

Other factors that can induce bronchoconstriction by activating the parasympathetic reflex include mechanical stimulation of the airway. For example, laryngoscopy, particles inhaled into the trachea or bronchi, as well as cold air inhalation, are all known to induce bronchoconstriction (Lumb, 2005).

In summary, the autonomic nervous system has the capacity to induce either complete airway closure or maximal dilation. Two physiologically distinct pathways differentially regulate airway tone by reflex control. Airway constriction is predominantly mediated by parasympathetic cholinergic activation whilst bronchodilation occurs with innervation of the NANC system. At rest tonically

active parasympathetic nerve activity provide a degree of immediately reversible airway tone that reflects a balance between contractile and relaxant parasympathetic activity (Canning & Fischer, 2001).

1.6.3. Airway Hyperresponsiveness in Asthma

Asthma is a disease of the airway characterised by airway inflammation, dyspnoea, cough, and bronchoconstriction in response to non-specific stimuli (West, 2003). Much of the research on airway biology in asthma has focussed on airway inflammation and its relationship with airway hyperresponsiveness. The predominant view in this regard has been that airway inflammation represented a causal mechanism with ASM providing the role of effecter (An *et al.*, 2007). Whilst chronic inflammation likely plays a key role in the development of airway hyperresponsiveness, through airway wall remodelling and the accumulation of excess muscle mass (Ebina *et al.*, 1990; Kips & Pauwels, 1999; Fernandes *et al.*, 2003), it has been shown recently that airway hyperresponsiveness and inflammation also manifest independently (Brusasco *et al.*, 1998; Crimi *et al.*, 1998). This has led to renewed interest for a more central role for ASM in airway hyperresponsiveness (An *et al.*, 2007).

The asthmatic airway is one that narrows too easily and too much (Woolcock & Peat, 1989). The most important end-effector of acute bronchoconstriction in asthma is considered to be the ASM, with excessive shortening of the smooth muscle cell considered to be the primary cause of excessive airway narrowing (Macklem, 1996; Lambert & Pare, 1997). Airways that narrow too easily and too

much in response to an airway challenge, are described as being hypersensitive and hyperresponsive, respectively (Woolcock & Peat, 1989). Airway hypersensitivity describes airways that respond to a contractile stimulus dose that would not provoke a response in a healthy individual. That is, the dose response curve is shifted to the left along the dose axis (Figure 1.9). Airway hyperresponsiveness refers to the extent that the airway smooth muscle shortens in response to contractile stimuli. When plotted as airway resistance versus dose, the healthy curve is typically sigmoid in shape, exhibiting a plateau response that indicates no additional shortening with further increases in stimulus magnitude. In asthma, the plateau is absent or elevated, indicating an increased propensity for the airway smooth muscle to shorten, with consequent increases in airway resistance (Figure 1.9).

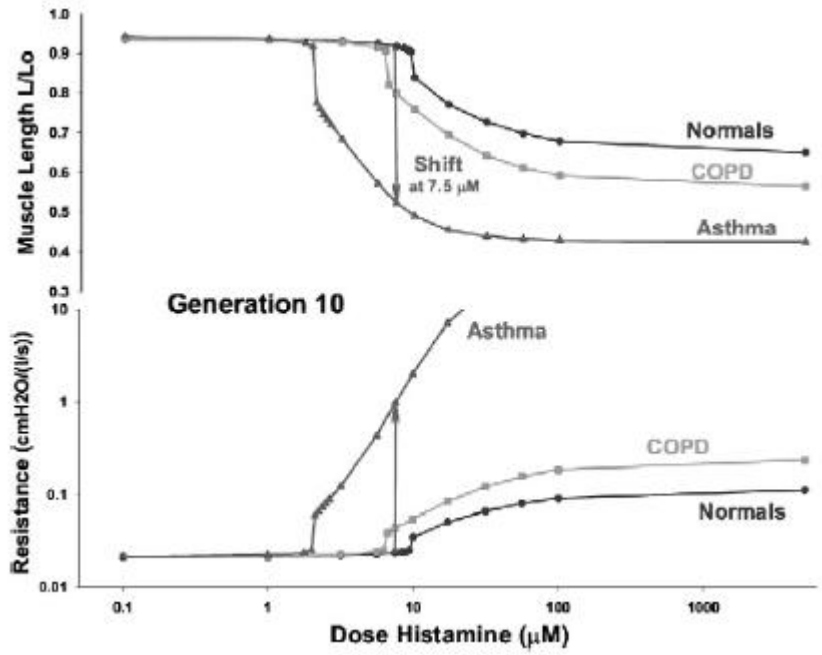

Figure 1.9. Airway muscle length and airway resistance in healthy, chronic obstructive pulmonary disease (COPD), and asthma, during increasing doses of contractile stimulus. From Mijailovich (2003).

The reason why smooth muscle in asthma narrows excessively is unclear but may be related to continual and irreversible remodelling of the airway wall in response to chronic inflammation (Fernandes *et al.*, 2003). Airway remodelling is associated with a number of structural changes to both the ASM and the respiratory epithelium and is seen with all degrees of asthma severity (Bergeron *et al.*, 2009). Associated changes in the airway include goblet cell and submucosal gland hyperplasia leading to increased sputum production. In addition, subepithelial fibrosis occurs in the peribronchial layer and leads to a thickening of the basement membrane. However, the primary mechanism for excessive airway narrowing is thought to be related to increased ASM mass from hypertrophy and hyperplasia of ASM cells (Lambert *et al.*, 1993; Pare *et al.*, 1997; McParland *et al.*, 2003; Fredberg, 2004). An alternative explanation is related to the elastic load that the ASM must shorten against. Both *in vivo* and *in vitro* studies have demonstrated that as the pre-load decreases, ASM shortens and airway narrowing increase (Gunst *et al.*, 1988). This suggests that in addition to the elastic load related to lung elastic recoil, the parenchyma also provides a resistance to airway deformation as a result of ASM shortening (Tepper *et al.*, 1999).

1.7. PLASTICITY OF THE LOWER AIRWAY

1.7.1. Response to Deep Inhalation

Evidence that implicates the involvement of ASM in asthma stems from research that has investigated the airway response to DI to TLC in patients with asthma compared to healthy individuals (Ingram, 1995; Pellegrino *et al.*, 1998b). Two responses to DI have been identified and are referred to as bronchoprotective and bronchodilatory. The bronchoprotective effect refers to the airway response to DIs taken **before** experimentally induced bronchoconstriction. The bronchodilatory effect describes the airway response to DIs taken **after** experimentally induced bronchoconstriction. A number of studies have investigated these responses and are discussed below.

Nadel and Tierney (1961) were the first to report the bronchodilating effect of a DI in healthy people. However, it was Fish *et al*. (1977) who first described that patients with asthma show a different response to a DI compared to healthy individuals and patients with other allergic conditions. Fish *et al*. (1977) compared the airway response to methacholine inhalation in patients with allergic rhinitis and with asthma. In the baseline state patients with asthma showed a gradual reduction in airway conductance (G_{aw}; reciprocal of resistance) following DI; further, a DI following methacholine-induced bronchoconstriction did not improve G_{aw}. Conversely, individuals with rhinitis showed a similar response to that seen in healthy people. Specifically, in the baseline state, a DI induced no change in G_{aw}, but following methacholine-induced bronchoconstriction, a DI was able to reverse the effect and improve G_{aw} (Fish *et al.*, 1977). The authors

proposed that the inability of patients with asthma to bronchodilate with a DI was an important factor in their airway responsiveness. Skloot *et al.* (1995) used maximal (FEV_1) and partial flow volume curves to investigate the dose response to inhaled methacholine in healthy and asthmatic individuals. The response to methacholine was similar in both groups when DI was prohibited (i.e. during the partial flow volume manoeuvre). Further, the airways of the healthy individuals remained hypersensitive to methacholine even after DIs were initiated. The authors argued that when increases in lung volume are suppressed, to minimise the modulating effect on airway tone, the healthy airway develops similar characteristics to the asthmatic airway. The authors concluded that a major feature of the hyperresponsiveness of asthma was an inability of inhalation to adequately stretch the airway smooth muscle (Skloot *et al.*, 1995). These observations were subsequently confirmed and extended by King *et al.* (1999a) who reported that an FEV_1, performed at the end of a methacholine dose response curve when DIs were prohibited, was significantly lower than that seen at the end of a dose response curve when DIs were taken periodically. The study design used by King *et al*. (1999a) also added further support to previous observations that indicated that a DI taken before an airway challenge in healthy individuals may also have a bronchoprotective influence. Malmberg and colleagues (1993) found that in healthy individuals the obstructive effect of single-dose provocations was highly dependent on the time interval between the administration of the contractile stimulus and the preceding spirometry. Specifically, the longer the interval between spirometry and stimulus, the stronger the effect of the stimulus. Kapsali *et al.* (2000) also reported a bronchoprotective effect of DIs in healthy subjects

and extended the findings to show that the effect was absent in individuals with asthma. The same group have gone on to suggest that the bronchoprotective effect of DI is more potent than the bronchodilatory effect and that the higher the number of DIs performed before the airway challenge the more effective the attenuation of any subsequent bronchoconstriction (Scichilone *et al.*, 2000; Scichilone *et al.*, 2001). Other studies in asthma patients and healthy individuals support the behaviour of ASM described above. King *et al*. (2001) reported that airway narrowing in asthma patients was greater following methacholine inhalation when DIs were prohibited for 15 min. These findings suggest that the stretch provided by DI is an important mechanism that may offer a protective effect to limit acutely induced airway narrowing. Wang *et al.* (2000) supported this assertion when they demonstrated that prior length changes in porcine ASM reduced its ability to generate tension and this, the authors suggested, may explain the mechanism responsible for the bronchoprotective effect of DI in healthy people. An absence of this refractory state of ASM in asthma may explain why the bronchoprotective effect of DI is diminished or absent (King *et al.*, 1999b). Using breath by breath measurement Jensen *et al.* (2001) showed that in healthy subjects R_{aw} at TLC was similar to baseline, even after provocation. However, patients with asthma not only dilated their airways less after DI at baseline and after provocation, but also showed greater re-narrowing compared to healthy subjects. This finding, as well as others discussed above suggests that ASM in asthma not only relaxes less in response to DI, it also appear to re-contract faster.

A number of proposals to explain the differential airway response to DI seen in asthma patients are reported in the literature. The first of these, based on the concept of airway and parenchymal hysteresis, was first proposed by Froeb & Mead (1968). This proposal is related to the observation that in asthma, not only is there an inability to reverse spontaneous obstruction with a DI, there is also a worsening of the bronchoconstriction (Burns *et al.*, 1985; Pichurko & Ingram, 1987). It is proposed that parenchymal hysteresis is greater than that of the airways during spontaneous asthma (Lim *et al.*, 1987; Lim *et al.*, 1989). In essence, this means that during inhalation, higher lung inflation pressures are required to overcome the elastic recoil forces of the lung, whereby deflation is characterised by much lower recoil pressures at all lung volumes. The forces that are transmitted to the airway via the parenchyma are directly proportional to the recoil pressure. Consequently, during deflation the traction applied to the airway by the parenchyma is diminished, leading to airways that are narrower and with higher resistance than they possessed prior to a DI. ASM tone is an important modulator of airway hysteresis that is influenced by the ability of ASM to respond to the stretch of a DI. Consequently, ASM tone is considered an important factor modulating how R_{aw} responds to the challenge of a DI (Burns *et al.*, 1985; Pliss *et al.*, 1989).

Fredberg and associates (1997; 1999; 2000) and Gunst *et al*. (1983; 1990) propose an alternative explanation for the abnormal response to DI seen in people with asthma. These authors argue that the forces transmitted to the ASM during normal tidal breathing represent an important mechanism that may affect airway

responsiveness. The theory of perturbed equilibria of myosin binding proposed by these authors argues that the strain associated with lung inflation during tidal breathing is transferred to the ASM. The mechanical effects are transmitted to the actin-myosin cross bridge interaction causing myosin to detach from actin (Fredberg *et al.*, 1999). Consequently, during tidal breathing, only a fraction of the total force-generating capacity of ASM is transmitted to the airway and the ASM remains compliant in what is described as a dynamically equilibrated contractile state (Fredberg *et al.*, 1997). In asthma, increased muscle mass coupled with lower force fluctuations result in stiffening of the ASM with a reduced ability to stretch (Gunst *et al.*, 1995; Fredberg *et al.*, 1997; Fredberg *et al.*, 1999). Thus, a vicious cycle develops whereby the reduced ability of ASM to stretch, stiffens the ASM, moving it towards what is described as a statically equilibrated 'latch' state, that is impervious to the perturbations of tidal breathing and DI (Fredberg *et al.*, 1999).

A number of studies, using a variety of techniques, have attempted to identify the mechanism(s) underlying the airway response to DI. Gunst (1983) subjected canine ASM tissue to tidal oscillations *in vitro*, which resulted in a decrease in contractile responsiveness. The authors proposed that stretch, induced by volume or load oscillations during tidal breathing, is a potential mechanism to reduce airway responsiveness. Other studies by the same group led them to suggest that mechanical stretch may alter the structure of the ASM cell such that its stiffness and contractility are reduced (Gunst *et al.*, 2003). Fredberg *et al*. (1997) extended the initial findings of Gunst suggesting that large tidal stretches of the ASM were

required to increase the hysteresis of ASM and dilate the airway. Further, it was suggested that if the tidal stretches lacked sufficient amplitude, ASM hysteresis would decrease and the ASM would subsequently becomes stiffer (Fredberg *et al.*, 1997). Muscle stiffness provides an indication of the number of actin-myosin interactions and it is proposed that when tidal stretches lack amplitude the amount of actin-myosin interactions increases and their rate of turnover is down regulated (Fredberg *et al.*, 1997). This results in the formation of slowly cycling latch-bridges that exhibit low hysteresis, high levels of stiffness, and low responsiveness to the stretch imposed by DI (Fredberg *et al.*, 1999). Airways that exhibit increased ASM mass due to airway wall remodelling are particularly susceptible to this outcome as are those that have had DIs prevented during bronchial challenge, or restricted, as in obesity (Brusasco *et al.*, 1999; Shore & Fredberg, 2005).

Another factor that may contribute to the magnitude of airway re-narrowing after a DI is the extent to which the airway dilates in response to the pressure generated by DI. Duggan *et al*. (1990) used chest wall strapping (CWS) to demonstrate that bronchodilation associated with DI was dependent on either achieving a lung volume > 69% of TLC, or by combining a lower lung volume (56% of TLC) with raised transpulmonary pressure as a consequence of CWS. The authors concluded that the size of the response to DI was primarily due to the ensuing transluminal airway pressure (Duggan *et al.*, 1990). Conflicting findings were published by Torchio *et al*. (2006) who found that CWS resulted in a reduction in the bronchodilatory ability of DI. However, methodological difference may have

accounted for the divergent results in this study, as the severity of CWS was substantially less than Duggan *et al*, limiting DI to approximately 88% of TLC. Consequently the transpulmonary pressures were substantially lower than the study of Duggan *et al* (~ 11 cmH_2O vs. ~ 19 cmH_2O).

Thus, the evidence suggests that a volume related stretch of sufficient magnitude or the achievement of a suitable transpulmonary pressure, or both, act to increase the hysteresis of ASM with subsequent reductions in stiffness and contractility. IMT provides a mechanism that involves repeated inhalation to near TLC concomitant with low transpulmonary pressures. Consequently, there is a clear theoretical basis for the suggestion that IMT might modulate the behaviour of ASM via volume and pressure related mechanisms.

1.7.2. Smooth Muscle Adaptation to Changes in Length

In contrast to skeletal muscle, the microstructure of ASM is disordered and in a continuous state of remodelling (Small & Gimona, 1998). Consequently, whilst the sliding-filament model of muscle contraction proposed by Huxley (1957), is useful to describe active force generation and shortening velocity of ASM, it is not useful when mechanical plasticity is considered (An & Fredberg, 2008). Smooth muscle functions over a far greater length range than skeletal muscle due to its role in allowing large volume changes in hollow organs such as the bladder and stomach (Wang & Pare, 2003). Consequently, force generation by ASM is independent of length as the number of contractile units varies in series, in response to length change (Pratusevich *et al.*, 1995; Ford, 2005). The immediate

response of ASM to increases in length is a reduction in force generating capacity that is proportional to the number, size, and frequency of the stretches (Wang *et al.*, 2000). Subsequent to the stretch-induced reduction in force production is a gradual recovery of force when the muscle is re-activated repeatedly at its new length (Figure 1.10; Wang *et al.*, 2001; Silberstein & Hai, 2002; An *et al.*, 2007).

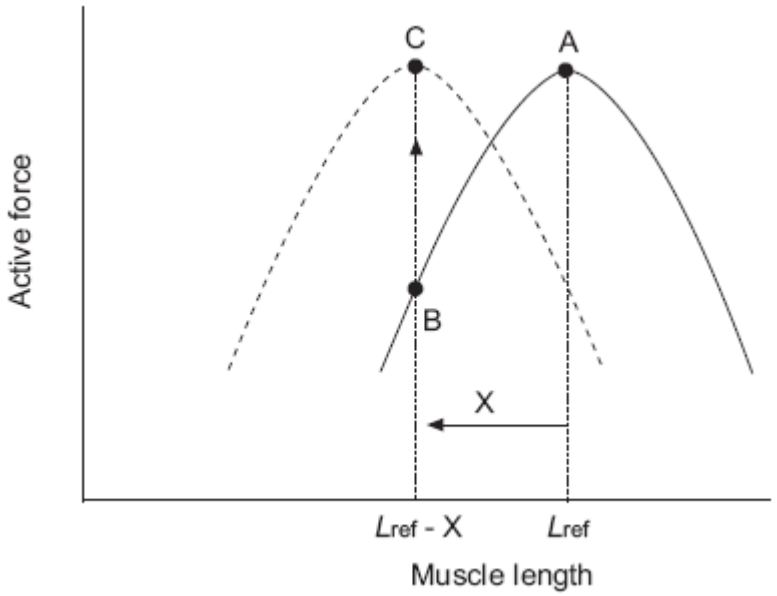

Figure 1.10. Length-force relationship of airway smooth muscle before length change (A); immediately after length change (B); and after length adaptation in response to repeated activation and relaxation (C). From An *et al.* (2007).

It is likely that the ability of ASM to adapt to changes in length stem from disassembly and reorganisation of the contractile apparatus to maintain optimal overlap of the contractile filaments (Seow, 2005). The ability of a single DI to remodel ASM by stretching the contractile apparatus acutely may explain the bronchoprotective effect of DI discussed previously. How length adaptation relates to the differential response seen in healthy and asthma patients is, however, unclear. It is possible that in asthma, the ASM has already adapted to a

permanently shortened state that progressively becomes more resistant to DI (Wang & Pare, 2003). Spontaneous asthma attacks may result in prolonged periods of airway narrowing in response to ASM activation caused by inflammatory mediator release. Further, oedema and vascular congestion of the airway wall may uncouple ASM from the parenchyma resulting in reduced tethering forces and prolonged periods of passive shortening of ASM (Wang & Pare, 2003).

In summary, it appears that the adaptation of ASM to a shorter length can lead to a chronic high-stiffness, low-hysteresis latch state (Fredberg, 2000). The likelihood of this outcome is enhanced when expansion of the chest wall is restricted; the ASM mass is increased, as well as any other factor that results in a reduced stretch of the ASM. However, this high degree of plasticity may also make ASM a highly responsive target for interventions that increase its resting length and reduce its stiffness.

1.7.3. Pressure-Threshold Inspiratory Muscle Training

Pressure-threshold IMT has traditionally been used as a tool to support the rehabilitation of patients with weakened thoracic respiratory muscles (McConnell & Romer, 2004). To initiate inhalation participants are required to generate a negative pressure of sufficient magnitude to overcome an inertial threshold load. The nature of the pressure-threshold load is such that it provides a quantifiable intensity with near flow independent resistance to inhalation (Caine & McConnell, 2000). A number of novel techniques have been used to achieve this,

including a spring-loaded poppet valve (Gosselink *et al.*, 1996; Caine & McConnell, 2000), a weighted plunger (Nickerson & Keens, 1982; Clanton *et al.*, 1985; Eastwood *et al.*, 1994) and a constant negative pressure system (Chen *et al.*, 1998).

Inspiratory muscle training in patients with asthma

As stated in section 1.6.3. the asthmatic airway is one that narrows too easily and too much. The mechanical factors associated with airway narrowing include, increases in flow resistive work, reduced dynamic lung compliance, and an increase in elastic work due to dynamic hyperinflation (Martin *et al.*, 1980). The consequences of dynamic hyperinflation include; 1) an increase in the work and oxygen cost of breathing, 2) functional weakening of the inspiratory muscles, and 3) a reduced ability to expand the tidal volume during exercise (O'Donnell *et al.*, 2007). It has previously been shown in people with mild asthma that dynamic hyperinflation is strongly associated with dyspnoea during acute methacholine-induced bronchoconstriction. Lougheed *et al.*, (1993) reported that 74% of the variance in the perception of breathlessness (Borg scale) could be attributed to changes in inspiratory capacity (an index of hyperinflation). An explanation for the increased perception of dyspnoea is likely related to the functional weakening of the respiratory muscles associated with hyperinflation. Functional weakening of the inspiratory muscles results in increased motor outflow for a given level of pressure generation and this manifests as an increased perception of dyspnoea (Leblanc *et al.*, 1988). In addition to functional weakening, there is also some evidence that the absolute strength of the inspiratory muscles may also be weaker

in people with asthma (Allen *et al.*, 1993; de Bruin *et al.*, 1997), although this is not a consistent finding (Stell *et al.*, 2001).

In view of the above, the rationale for IMT is that by improving the maximum capacity of the inspiratory muscles for pressure generation, a lower percentage of that maximum would be required with each breath. Evidence supporting reduced motor drive to the respiratory muscles post IMT has been reported by Huang *et al.* (2003). These authors reported a 22% reduction in inspiratory motor drive (mouth occlusion pressure at 0.1 s; [$P_{0.1}$]) after 4 weeks of IMT that increased MIP by 36% (Huang *et al.*, 2003). Although improvements in MIP only accounted for 21% of the variation in $P_{0.1}$, suggesting that other factors are involved, the Huang study does provide supporting evidence for IMT induced reductions in inspiratory motor drive. Reductions in motor drive such as this may have the potential to positively influence the perception of dyspnoea in patient with asthma.

Despite the above there is a paucity of good quality randomised controlled trials investigating IMT in patients with asthma. However, some studies have shown an association between perception of dyspnoea, consumption of β_2-agonists, and inspiratory muscle strength (Weiner *et al.*, 2002a; Weiner *et al.*, 2002b) and this relationship appears to be modulated by IMT. Specifically, the perception of dyspnoea to a loaded breathing task was significantly higher in patients who had a high consumption of β_2-agonists compared to those who had a normal consumption ($\leq$ 1 puff/day). Further, improvements in MIP after 3 months IMT were associated with statistically significant reductions in the mean Borg score

during loaded breathing ($P < 0.05$), as well as in the mean daily β_2-agonist consumption (Weiner *et al.*, 2000a).

Despite the evidence presented above a Cochrane review (edited, with no change to the conclusions, in 2009) concluded that there was insufficient evidence to state that improvements in MIP provided a measurable clinical benefit to people with asthma (Ram *et al.*, 2003). The report also highlighted that the majority of studies have come from the same research group (Weiner *et al.*, 1992; Weiner *et al.*, 2000a; Weiner *et al.*, 2002a; Weiner *et al.*, 2002b) and suggested that further studies are warranted to confirm their findings.

Aside from improvements in inspiratory muscle strength, endurance, and exertional dyspnoea, some studies have reported improvements in pulmonary function in patients with asthma (Weiner *et al.*, 1992; McConnell *et al.*, 1998). Specifically, improvements in forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and peak expiratory flow rate (PEF) have been reported for short (3 week) and long duration (6 month) interventions that also reported improvements in inspiratory muscle strength. The mechanism by which improvements in lung function may occur with IMT is unclear, but may be related to repeated stretch of the ASM due to increases in transluminal airway pressure. Also, the ability to achieve a greater lung volume after IMT, due to stronger inspiratory muscles, might result in a larger stretching effect of the airway due to volume related parenchymal pull. Both of these mechanisms might together, or independently, result in reductions in R_{aw}.

Of specific interest in the context of the current thesis is the effect that the inertial properties of a threshold load may have on ASM. Because the threshold valve does not open until intrathoracic pressure equals the valve opening pressure, there is a brief period, prior to valve opening, when the space proximal to the valve is decompressed. This includes the intrathoracic space and it is likely that decompression of this space has a brief stretching effect on the airway that may induce relaxation of ASM. In addition, IMT is typically associated with twice daily periods of deep inhalation that approach TLC. Consequently, there may be a combined influence upon the ASM from pressure and volume related mechanisms.

1.8. ASSESSMENT OF LOWER AIRWAY FUNCTION

The ability to ventilate the lungs more rapidly and at higher volumes than required at rest is essential for everyday activity and exercise. Any reduction in the capacity for increasing flow and volume can cause breathlessness and may limit functional capacity (Quanjer *et al.*, 1993). The existence and severity of airflow obstruction can be assessed by the measurement of respiratory system resistance during tidal breathing as well as forced expiratory flows and volumes (Pellegrino *et al.*, 1998b). The most common, and readily accessible of these techniques, is the measurement of forced expiratory volumes and flows, obtained from the maximal expiratory flow-volume curve (MEFV). Of the indices derived from the MEFV the FEV_1 is considered to be the most important in the determination and monitoring of asthma (Quanjer *et al.*, 1993). However, it has been known for some time that performing a maximal expiratory manoeuvre can influence the degree of airway obstruction and this is most likely due to the deep inhalation taken prior to expiration (Pellegrino *et al.*, 1998b). Consequently, careful consideration needs to be given to the choice of technique when assessing airway function.

1.8.1. Spirometry

Spirometry is a physiological test that is, perhaps, the most widely available non-invasive test of pulmonary function (Burgel *et al.*, 2009). Spirometry measures inhaled and exhaled volumes of air as a function of time, giving a primary signal for either volume (L) or flow ($L \cdot s^{-1}$). Plotting either volume against time, or flow against volume provides a means of visually assessing lung function that provides

additional information of the status of airway function. Flow-volume loops were first introduced by Hyatt *et al.* (1958) and are typically generated by performing a maximal expiratory effort that results in the measurement of the FVC. The FVC is defined as the total volume of air measured from a maximal and complete forced expiration, starting from a full inhalation and consists of three distinct phases: 1) maximum inhalation; 2) a 'blast' of exhalation; and, 3) continued and complete exhalation (Miller *et al.*, 2005). An example of a flow-volume loop from a healthy participant is displayed in figure 1.11 A, below.

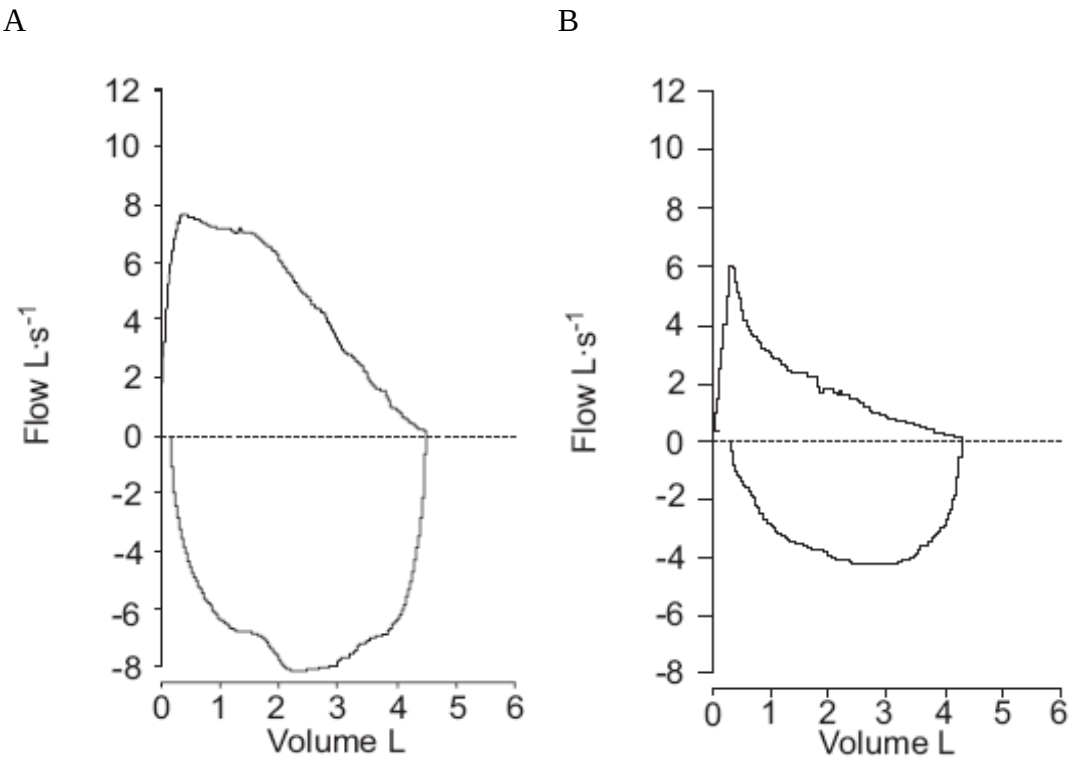

Figure 1.11. Flow-volume loops of a healthy subject (A) and a patient with asthma (B) exhibiting moderate airflow obstruction From Miller *et al.* (2005).

The basic parameters used to accurately interpret lung function are the FVC, the FEV_1, the FEV_1/FVC ratio and, the TLC. All, except TLC, can be measured with simple spirometry. The FEV_1 is the volume of air expired in the first second of the

FVC manoeuvre and is largely influenced by the larger proximal airways (Burgel *et al.*, 2009). The start of test (time zero) is standardised for all timed measurements by the back extrapolation method, ensuring, in the event of a delayed start, that the back extrapolated volume is less than the greater of 5% of the FVC or 0.15 L (Miller *et al.*, 2005).

The PEF occurs during the early phase of a forced expiration and is defined as the highest flow rate achieved during the FVC manoeuvre (Miller *et al.*, 2005). PEF is highly dependent on effort as well as the lung volume that the manoeuvre is initiated from. Consequently, the measurement of PEF, whilst suitable for asthma-management and self-monitoring, is not recommended for diagnostic purposes (Quanjer *et al.*, 1993).

The mean forced expiratory flow (FEF) between 25 and 75% of the FVC ($FEF_{25-75\%}$) and the forced expiratory flow at 50% of FVC ($FEF_{50\%}$) are both indices of expiratory flow through the middle lung volumes. Both measures have been proposed to reflect the function of the more distal airways, but have been shown to be highly variable measures (Hansen *et al.*, 2006; Burgel *et al.*, 2009). Further, the utility of mid-range expiratory flows in monitoring reversibility to bronchodilator or response to airway challenge has been called into question (Dickinson *et al.*, 2006; Burgel *et al.*, 2009). The difficulty in interpreting changes in $FEF_{25-75\%}$ and FEF_{50} are mainly due to the dependence of the measure on FVC. Consequently, if FVC changes between measurements the FEF values are no

longer comparable. Burgel *et al.* (2009) suggests that the measures are of limited reliability and caution should be used in interpreting the measurement.

Reliability

A number of factors may contribute to the variability of spirometric measurement. Aside from instrument errors, which can be minimised with careful validation and calibration, and errors attributed to how the investigator conducts and interprets the test, the major source of variability is biological. Factors that may influence lung function include diurnal variability, exposure to physical or chemical stimuli as well as the performance of the forced expiratory manoeuvre itself (Quanjer *et al.*, 1993). Consequently, tests should ideally be conducted by the same investigator, at the same time of day, and with the same equipment (Miller *et al.*, 2005). Further, it is important that the performance of the manoeuvre is standardised to ensure that the volume history is consistent between measurements (Pellegrino *et al.*, 1998a). For example, it is recommended that the inhalation to TLC is continuous and smooth, with the subsequent maximal exhalation initiated with minimal pause (Quanjer *et al.*, 1993). To ensure the validity and reproducibility of spirometric measurements it is recommended that all tests are undertaken in accordance with guidelines published by the European Respiratory Society and the American Thoracic Society (Miller *et al.*, 2005).

Suitability of spirometry to assess airway responsiveness

The principal factors that determine the FVC are the force exerted by the expiratory muscles during the manoeuvre, the elastic recoil force of the lung, and

the patency of the airways (Quanjer *et al.*, 1993). Pleural and alveolar pressures are greatly increased above pressure at the mouth during the FVC manoeuvre, although after a short effort dependent phase, pressure from the alveoli to the mouth drops, resulting in lower intra-thoracic pressure compared to pleural pressure (Quanjer *et al.*, 1993). The impact of this is that subsequent to the brief effort dependent phase the airways become dynamically compressed, flow limiting, and effort independent (Quanjer *et al.*, 1993). Flows recorded during the early, effort dependent, phase of a maximal expiration are mostly affected by the proximal airways, with the distal airways contributing mostly to flows measured during the effort independent phase of expiration (Lapp & Hyatt, 1967). Any pathology affecting the determinants of the FVC manoeuvre will influence the FVC measurement. For example, asthma is characterised by a disproportionate decrease in maximal expiratory flow in relation to the FVC (Pellegrino *et al.*, 2005). The reduced airflow is due to airway narrowing throughout exhalation and is defined by an FEV_1/FVC ratio below the 5^{th} percentile of the predicted value (Pellegrino *et al.*, 2005). The characteristic concave shape of the flow-volume loop due to slowing of expiratory flow can be seen in figure 1.11 B. Airway obstruction in asthma is due to inflammation and smooth muscle contraction (Lumb, 2005).

The airway response to an intervention such as a bronchodilator, or in the context of this thesis, IMT, can be assessed either as an acute dose response or after a chronic intervention lasting a number of weeks (Pellegrino *et al.*, 2005). There is no agreement about what constitutes bronchodilation in patients with airway

obstruction and this is confounded by a lack of consensus on how to express the bronchodilator response (Pellegrino *et al.*, 2005). In view of this, common approaches have been to use the absolute change, percent of the initial value, and the percent of predicted to determine the effect of an intervention. When expressing the value as a percentage of the initial value a meaningful response needs to exceed the variability of the measurement, which is typically > 8%, or approximately 150 mL (Brand *et al.*, 1992). Current recommendations propose that both the percent change from baseline and the absolute change in FVC and FEV_1 are used to determine a positive response (Pellegrino *et al.*, 2005). A clinically significant bronchodilation is considered to be apparent when relative and absolute values exceed 12% and 200 mL, respectively. However, it is worth considering that the absence of a significant bronchodilator response does not necessarily rule out a clinically meaningful effect (Redelmeier *et al.*, 1996). One of the reasons to explain this is that the use of maximal forced expiratory manoeuvres to determine changes in lung function after an intervention intended to improve airway calibre is sometimes considered to be problematic (Pellegrino *et al.*, 2005). For example, compared to the measurement of airway resistance, FEV_1 and FVC often underestimate the response to bronchodilator therapy (Pellegrino *et al.*, 1998a). This is most likely due to the effect of the preceding deep inhalation at the start of the manoeuvre and the positive effect this has on airway calibre, particularly after administration of a bronchodilator (Wang *et al.*, 1990). This makes monitoring the effect of a DI, or an acute bout of IMT, difficult to determine as the manoeuvre assessing the response is likely to modulate airway calibre potentially masking any effect. An alternative method is to assess lung

function parameters within the tidal breathing range, which negates the requirement for a preceding deep inhalation. An estimate of airway calibre that is less affected by volume history can be measured from the forced expiratory flow from a partial inflation at approximately 60% FVC (Barnes *et al.*, 1981). By comparing partial (P) and maximal (M) expiratory flows (M/P) at a specified lung volume an indication of the effect a deep inhalation has on airway calibre can be determined (Figure 1.12; Pellegrino *et al.*, 1998b). An M/P > 1 is due to a greater expiratory flow on the maximal loop at the specified lung volume and suggests that the preceding deep inhalation had a bronchodilator effect. Conversely, an M/P < 1 indicates that expiratory flow at the specified lung volume is less on maximal loop and suggests that bronchoconstriction has occurred (Pellegrino *et al.*, 1998b).

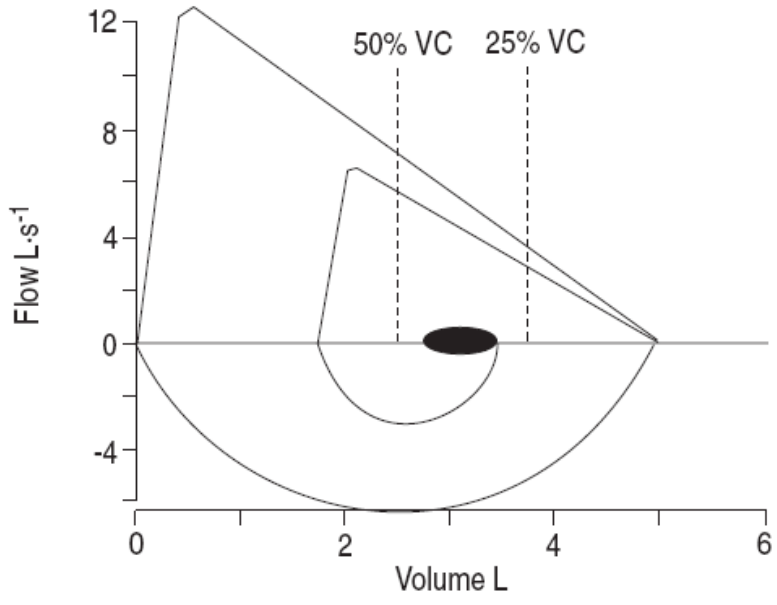

Figure 1.12. Schematic of partial and maximal flow-volume curves. The volume that the partial forced expiration is initiated is approximately 60% of VC. The dotted lines represent the lung volume at which the maximal to partial flow ratio (M/P) is calculated. From Pellegrino *et al.* (1998b).

In the context of this thesis spirometry provides a useful tool to determine the general lung function of potential participants. Further, spirometry can also be used as a screening tool to assess the suitability of participants for study selection based on the degree of their airway obstruction. In view of the above it is appropriate to include spirometry as a method to monitor potential changes in airway calibre due to IMT. However, due to the potential for underestimating any improvements in lung function, and the unsuitability of forced expiratory manoeuvres to monitor the effect of a previous DI, an alternative method of assessing changes in airway calibre is needed.

1.8.2. Forced oscillation technique

The forced oscillation technique (FOT) is a non-invasive method used to assess respiratory mechanics that, unlike spirometry, requires only minimal patient involvement in the measurement. An advantage of the FOT is that there is no requirement for forced respiratory manoeuvres that may affect airway smooth muscle tone and consequently respiratory mechanics (Navajas & Farre, 2001). This makes the FOT a particularly attractive method for assessing changes in airway calibre in response to deep inhalation or airway challenge tests.

The FOT determines the mechanical properties of the respiratory system via externally applied pressure signals (~ 1cmH_2O) and the resultant flows. When pressure oscillations are applied at the mouth at a high enough frequency (> 2 Hz) the activity of the respiratory pump muscles, which operate at approximately 0.2 Hz at rest, becomes negligible and independent of the participant's breathing

pattern. Consequently, the only pressure acting at the FOT frequency is that applied by the device itself and this, alongside the measurement of flow rate, can be used to assess the mechanical properties of the respiratory system. The key concept behind the FOT in terms of respiratory system mechanics is the measurement of impedance (Z_{rs}; Oostveen *et al.*, 2003). Impedance encompasses the parameters resistance and the less familiar measurement term, reactance. Respiratory system resistance (R_{rs}) represents the pressure-flow relationship of the pressure oscillation that is 'in phase' with airflow whilst respiratory system reactance (X_{rs}) describes the 'out of phase' relationship that represents the opposing elastic and inertial forces of the respiratory system (Goldman, 2001). In simple terms R_{rs} represents the dissipative properties of the respiratory system and X_{rs} relates to the capacity of the respiratory system for energy storage (Oostveen *et al.*, 2003). The oscillation frequency where the opposing reactive forces are equal is termed the resonant frequency (Fres) and at this frequency X_{rs} is equal to zero (Goldman, 2001). The relationship between R_{rs}, X_{rs} and oscillation frequency in healthy individuals and patients with obstructed airways is shown in Figure 1.13. The flow induced by the pressure oscillations emitted from the FOT device has amplitude that is inversely proportional to the impedance of the respiratory system. This means that airways that are obstructed are associated with lower flows at a specified oscillatory pressure (Navajas & Farre, 2001). Changes in Z_{rs} that are characteristic of airway obstruction are due to increases in R_{rs} and a decrease in X_{rs} concomitant with an increase in F_{res} (Figure 1.13).

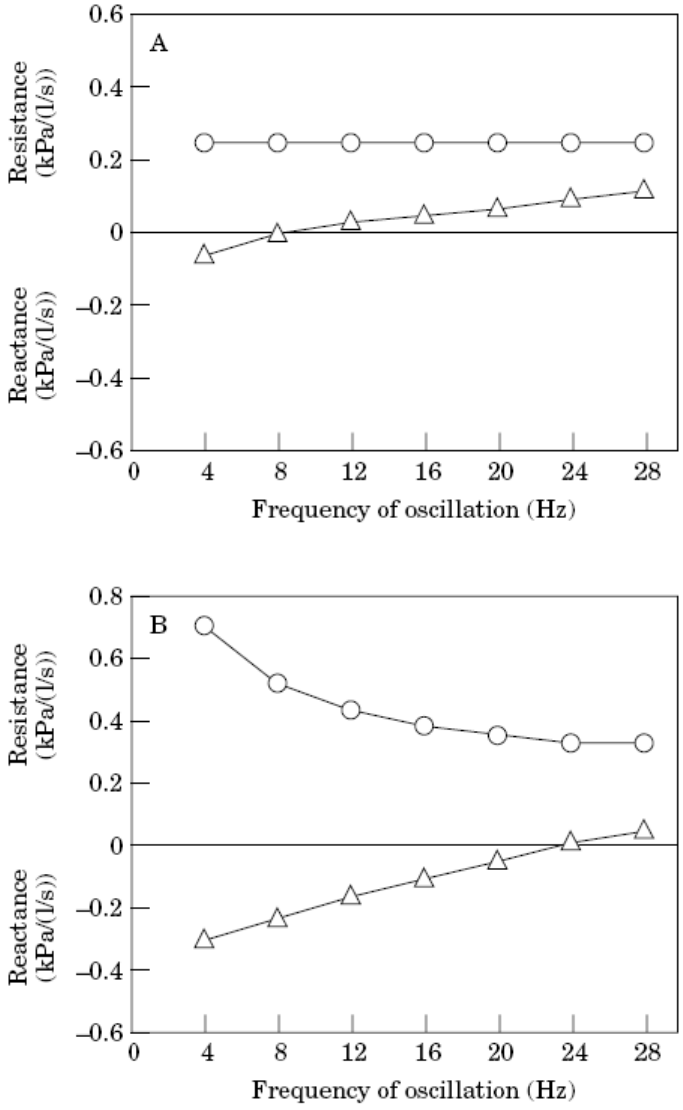

Figure 1.13. Schematic of resistance and reactance at different oscillation frequencies in healthy adults (A) and patients with airway obstruction (B). The frequency dependence of R_{rs} in airway obstruction is clearly apparent in panel B, as is the negative reactance that does not reach resonant frequency until 24 Hz. From Goldman (2001).

In the medium frequency range (4 – 48 Hz) R_{rs} in the healthy adult is largely independent of the frequency at which it is measured. However, this is not the case for individuals with obstructive pulmonary disease who exhibit a marked frequency-dependence of resistance (Grimby *et al.*, 1968). In essence, the higher the oscillation frequency the closer resistance to airflow comes to normal values. Consequently, R_{rs} is typically specified at lower frequencies to ensure differences

between healthy and patient groups are not masked (Figure 1.13; Goldman, 2001). In the lower frequency range of 5-10 Hz it is suggested that R_{rs} largely reflects airway resistance (R_{aw}; Hantos *et al.*, 1992). Jensen *et al*. (2001) are more specific and suggest that a frequency of 8 Hz better reflects R_{aw} and should be utilised when tracking changes in airway calibre.

The first study to use the FOT to compare R_{rs} of healthy individuals and patients with lung disease was undertaken by Fisher *et al.* (1968). In healthy subjects the mean R_{rs} was reported to be 2.3 $cmH_2O \cdot s \cdot L^{-1}$ whilst in individuals with obstructive lung disease R_{rs} was 8.1 $cmH_2O \cdot s \cdot L^{-1}$. Further, Clement *et al.* (1983) reported that the FOT had the sensitivity to distinguish healthy individuals from patients with respiratory complaints irrespective of whether they had reduced forced expiratory flows.

Reliability

A number of studies have assessed the short- and long-term intra-individual variability in R_{rs} assessed by the FOT in both healthy individuals and patients with airway disease. Neild *et al.* (1989) determined the short-term, intra-individual reproducibility of R_{rs} measured at 10 Hz in healthy individuals and patients with asthma. A coefficient of variation (CV) of 11.3% and 10.3% was reported in the healthy and patient groups, respectively. Similar values were reported by van den Elshout *et al.* (1990) who reported a CV of 8.3% in healthy subjects and 10.0% in patients with asthma for R_{rs} measured at 8 Hz. The same authors also reported data for between-day variability in the same subjects. They found that the

between-day variation in R_{rs} was slightly more than that for within-day variation with a CV of 10.1% in healthy individuals and 11.5% in patients (van den Elshout *et al.*, 1990). Further, R_{rs} assessed by FOT shows a strong agreement with other indices of airway calibre such as plethysmography and spirometry (Pairon *et al.*, 1994; Oostveen *et al.*, 2003).

Suitability of FOT to assess airway responsiveness

Changes in lung function parameters in response to airway challenge or reversibility testing rely somewhat on the variability of the measurement. A positive effect or response is typically one that exceeds the baseline CV by a factor of two (Oostveen *et al.*, 2003). Although the resolution of FOT is enhanced at lower frequencies in terms of identifying peripheral airway disease lower frequencies are subject to the greatest intra-individual variability (see above). This is likely due to the closeness these frequencies have with that of the natural breathing cycle (Goldman, 2001). Significant reductions in R_{rs} have been reported in response to bronchodilator that has also been associated with improvements in FEV_1 (van Noord *et al.*, 1994). Further, it was reported that post- bronchodilator reductions in R_{rs} at 6 Hz were significantly correlated with FEV_1 (van Noord *et al.*, 1994). However, these authors concluded that FOT was noticeably less sensitive as an indicator of a bronchodilator effect than spirometry. In contrast Zerah *et al.* (1995) came to the opposite conclusion stating that FOT was a suitable technique for determining the degree of bronchodilation in patients. The contrast between the two studies may be related to the severity of airway obstruction in some of the patients in the van Noord study. As such, a

considerably higher threshold value was chosen to represent a significant bronchodilation in the van Noord study, compared to Zerah *et al*. (> 45% vs. 10% decrease in R_{rs}). Consequently, it would appear that in well-defined groups with similar degrees of airway obstruction the FOT is a suitable and equivalent technique for assessing the bronchodilatory response (Oostveen *et al.*, 2003).

It has also been shown that FOT provides a more sensitive index than FEV_1 to detect changes in bronchial hyperresponsiveness. Pennings & Wouters (1997) assessed the effect of 6 wk corticosteroid treatment on cold air isocapnic hyperventilation. A significant attenuation (-26%) of R_{rs} at 8 Hz was observed in the absence of any changes in spirometry. The FOT proved to be a more sensitive measure of changes in bronchial tone that may be explained by the effect of the DI preceding the forced expiration on smooth muscle tone.

Potential changes in airway tone in response to acute or chronic IMT are likely to occur in the distal airways. Detection of changes in R_{rs} that largely reflect airway resistance of the distal airways requires FOT frequencies of approximately 8 Hz (Jensen *et al.*, 2001). Unfortunately, at this resolution FOT becomes increasingly less reliable due to the reasons discussed above. However, despite this limitation it seems appropriate, in the context of this thesis, to use a technique that complements spirometry and does not influence the outcome that is being measured.

1.9. SUMMARY

The information provided in this chapter suggests that current research on the effect IMT may have on the airway is either absent or conflicting.

A clear rationale has been presented to support the notion that the pharyngeal UA dilator muscles might be activated during IMT. In addition, evidence has been provided that demonstrates that the UA muscles exhibit both adaptive and maladaptive responses to stimulus in health and disease, respectively (Series *et al.*, 1996; Vincent *et al.*, 2002). Also, positive outcomes of the effect of IMT on laryngeal function have been reported (Baker *et al.*, 2003b; Dickinson *et al.*, 2007), although it is acknowledged that the evidence base in this respect is somewhat weak, due to a predominantly single subject, case study design.

A rationale has also been presented to support the argument for IMT induced improvements in lung function. Gunst *et al*. (1988) proposed that the opening of narrowed airways may require more negative pressures than those normally generated by DI. More recent evidence has suggested that positive pressure inflation may provide a stretch to the ASM that reduces airway obstruction (Slats *et al.*, 2008). If IMT was able to impart a stretching force upon the airway in excess of that achieved under physiologic conditions it may explain the improvements in lower airway function have been shown by some authors (Weiner *et al.*, 1992; McConnell *et al.*, 1998; Lima *et al.*, 2008).

1.10. AIMS AND OBJECTIVES

In view of the above and based on the literature reviewed the principal aims of this thesis are:

1. To determine whether primary upper airway dilator muscles are activated by an acute bout of pressure-threshold IMT.

2. To examine the effect of IMT on upper airway narrowing during loaded breathing.

3. To determine the acute effect of various inspiratory pressure-threshold loads on respiratory system resistance in people with moderate asthma.

4. To investigate the effects of IMT on airway function, baseline respiratory system resistance, and response to deep inhalation in people with moderate asthma.

CHAPTER TWO

GENERAL METHODS

2.1. PRE-TEST PREPARATION

2.1.1. Ethical Approval

Ethical approval was obtained from Brunel School of Sport and Education Research Ethics Committee, a sub-group of Brunel University Ethics Committee, prior to all studies contained within this thesis (See Appendix A-1).

2.1.2. Participants

Written informed consent and completion of a general health questionnaire were required prior to testing (See Appendix A-2 and A-3). In addition, detailed information sheets were provided to all participants prior to commencement of any testing. Any participants who had experienced a respiratory tract infection within two weeks prior to testing were excluded from the studies due to the potential effects upon lung and respiratory muscle function (Mier-Jedrzejowicz *et al.*, 1988; Quanjer *et al.*, 1993). Participants were familiarised with all testing procedures prior to formal testing. On testing days, participants were requested to abstain from alcohol and caffeinated beverages and any other substance known to, or suspected of, affecting normal human physiological function. Participants were also asked to refrain from eating in the two hours prior to testing and to abstain from physical activity in the 24 hrs prior to testing.

Details of specific inclusion criteria are detailed in the relevant chapters.

2.1.3. Testing Conditions

Environmental conditions were not standardised, although testing sessions were scheduled for a similar time of day (± 1 h) to minimise the effect of diurnal

biological fluctuation (Atkinson & Reilly, 1996). All tests were arranged to minimise any effect a prior test may have upon subsequent tests.

2.2. EQUIPMENT AND PROCEDURES

The general methods used in this thesis are described in the following sections. More specialised equipment and procedures are described in the relevant chapters.

2.2.1. Anthropometry

Stature was measured to the nearest 0.001 m with a standard stadiometer (Model 798, Seca Ltd, Birmingham, UK). Specifically, participants were required to stand straight with their back, buttocks and heels against the wall. The participant's head was manually oriented into the Frankfort Plane. After taking a deep breath and stretching upward the Broca plane was lowered to the vertex of the head. The maximum distance from the floor to the vertex was recorded. Body mass in minimal clothing was recorded to the nearest 0.1 kg on calibrated electronic scales (Model 798, Seca Ltd, Birmingham, UK).

2.2.2. Spirometry

Spirometry was assessed in the sitting position using an electronic flow-measurement spirometer (Microloop, Micro Medical Ltd, Rochester, Kent, UK) connected to a PC running dedicated software (Spida 5, Micro Medical Ltd, Rochester, Kent, UK). The spirometer incorporated a turbine flow meter that counted the number of interruptions of a light beam by a lightweight vane. The participant's inspiratory and expiratory flow caused the vane to turn at a rate proportional to the rate of airflow, and this signal was converted to flow in litres per second. Volume was computed automatically by time integration. The accuracy and precision of the spirometer was in accordance with the requirements

of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) (Miller *et al.*, 2005). Calibration of the equipment was checked daily using a 3 L calibration syringe (Calibration Pump, Jaeger, Hoechberg, Germany). All measurements were performed and interpreted according to ATS/ERS guidelines (Miller *et al.*, 2005; Pellegrino *et al.*, 2005). Specifically, a minimum of 3 acceptable forced vital capacity (FVC) manoeuvres were performed. Acceptable manoeuvres were free from artefact and had a satisfactory start and end of test. On completion of three acceptable tests the results were assessed for repeatability. Specifically, if the difference between the largest and next largest FVC and forced expiratory volume in 1 s (FEV_1) was $\leq$ 0.15 L the testing was terminated. If repeatability criteria were not met, testing continued up to a maximum of eight manoeuvres. The largest FVC and FEV_1 were selected from all the useable tests with other indices such as peak (PEF) and mid (FEF_{25-75}) expiratory flows, derived from the test with the largest sum of FVC and FEV_1. Figure 2.1 describes the process of how acceptability and repeatability criteria were applied.

A demonstration of correct technique was carried out by the test experimenter prior to testing to ensure participants were fully aware of the requirements of the test. Participants performed the manoeuvres via a mouthpiece that incorporated a filter to minimise the risk of infection as well as contamination of the equipment. A nose clip was worn to prevent leaks through the nares. Participants were instructed to inhale rapidly and completely from functional residual capacity (FRC) to TLC. From TLC participants were then instructed to 'blast' the air from their lungs with minimal hesitation (< 2 s), and to complete a full exhalation. On

reaching maximal expiration participants performed a maximal forced inspiratory effort to TLC. Verbal encouragement was given throughout the manoeuvre.

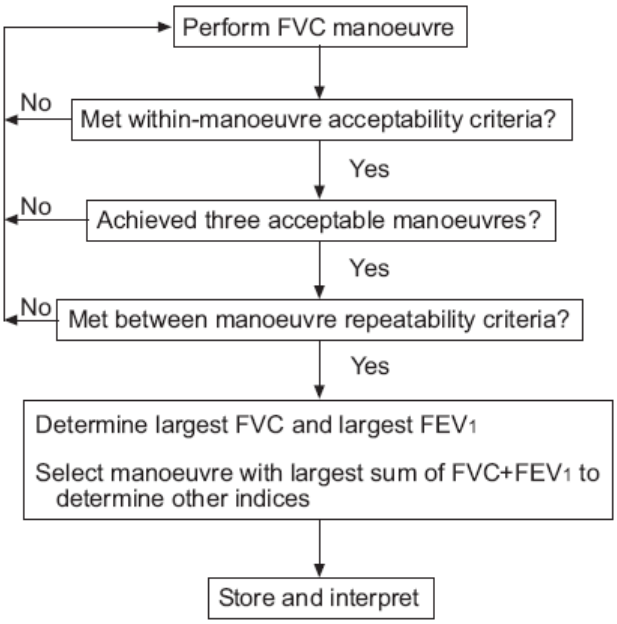

Figure 2.1. Application of acceptability and repeatability criteria to forced expiratory manoeuvres. FVC: forced vital capacity; FEV_1: forced expiratory volume in one second. (from Miller *et al.*, 2005).

2.2.3. Respiratory Muscle Function

Maximum inspiratory mouth pressure (MIP) and maximum expiratory mouth pressure (MEP) were determined from residual volume (RV) and TLC, respectively, using a portable hand held mouth pressure meter (Micro RPM, Micro Medical Ltd, Rochester, Kent, UK). A leak of 1 mm diameter was incorporated within the pressure meter to prevent closure of the glottis and the generation of artificially high inspiratory pressures (Black & Hyatt, 1969). Participants performed the manoeuvres via a flanged mouthpiece with a nose clip in position to prevent leaks through the nares. A demonstration of correct technique was carried out by the test experimenter prior to testing to ensure

participants were fully aware of the requirements of the test. Participants performed the manoeuvre seated and were strongly encouraged to make maximum inspiratory (Mueller manoeuvre) and expiratory (Valsalva manoeuvre) efforts throughout the test. As MIP and MEP measurements are dependent upon lung volume, each effort was initiated from TLC or RV, respectively. During the expiratory efforts participants pinched their lips around the mouthpiece and supported the cheeks to minimise the use of buccal muscles. Each manoeuvre lasted for a minimum of 1.5 s to ensure that the maximum average pressure sustained over 1 s could be recorded (Figure 2.2).

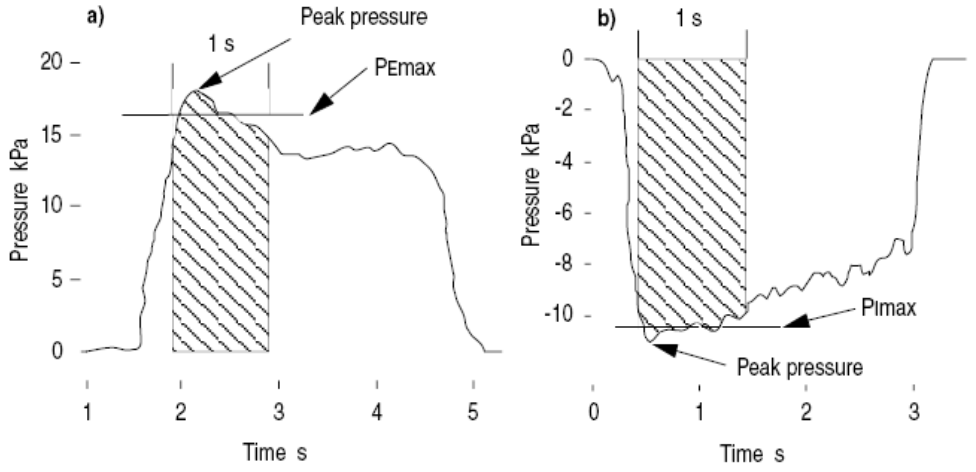

Figure 2.2. A typical pressure trace of maximal expiratory (a) and inspiratory (b) efforts recorded using a hand-held portable mouth pressure meter. From Hamnegård *et al.* (1994).

The device reports the maximum average pressure sustained over 1 s within the range of ± 300 cmH_2O. Hamnegård *et al.* (1994) confirmed the precision and accuracy of hand held pressure meters. When compared to a research standard differential pressure transducer, these authors reported a mean ± SD difference of 1.9 ± 1.2 and -0.4 ± 1.2 cmH_2O for MIP and MEP, respectively. A maximum of

10 manoeuvres were attempted with 60 s recovery between each. The maximum average pressure sustained over one second from three manoeuvres that varied by less than 10% was recorded. This requirement is more stringent than that required of current guidelines, which suggest that 20% is adequate (ATS/ERS, 2002).

2.2.4. Inspiratory Muscle Training and Adherence

Inspiratory muscle training was undertaken using a commercially available pressure-threshold training device (POWERbreathe®, HaB Ltd, Southam, UK; Caine & McConnell, 2000; Figure 2.3).

Figure 2.3. The POWERbreathe® inspiratory muscle training device. From Caine & McConnell (2000).

At the start of the training period the inspiratory load was set to 50% of MIP. The desired pressure was achieved by adjusting the degree of spring compression in

the device whilst inspiratory pressure was simultaneously recorded via a fine bore needle inserted into the mouthpiece and connected via tubing to a mouth pressure meter. Participants were given instructions to perform 30 maximal dynamic inspiratory efforts, from RV to TLC, twice daily for six weeks. This load has previously been shown to result in improvements in MIP of approximately 30% (Romer *et al.*, 2002b, a; Romer & McConnell, 2003). Participants were also instructed to periodically increase the load to a level that would only just allow them to complete the 30 manoeuvres. To monitor compliance with the training programme, participants completed a self-report training diary for the duration of the training period.

In light of recent evidence reporting a learning effect on MIP (Terzi *et al.*, 2010) it is pertinent to note that all participants who took part in the studies described in chapter 3 and chapter 4 were familiar with MIP and MEP measurements and had performed them on numerous occasions. Consequently, it is likely that the values reported are a true representation of inspiratory and expiratory muscle function. Therefore, the baseline value used to determine the initial IMT load in chapters 3 and 4 is considered to be valid. The participants who took part in the studies described in chapters 5 and 6 were naïve to the measurement of MIP and MEP. Therefore, it is possible that the reported baseline values underestimate MIP. Consequently, the training load for IMT may have been lower than the recommended 50% MIP. However, it is important to note that the learning effect reported by Terzi *et al.*, (2010) amounted to an improvement in MIP of 4 cmH_2O (97 ± 28 vs. 101 ± 30 cmH_2O). A similar underestimation of MIP in the

participants described in chapters 5 and 6 would have meant that the IMT training load may have been set approximately 2 cmH_2O below the optimal level. The functional significance of this is likely to be minimal.

2.2.5. Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) was undertaken in accordance with the guidelines set out by the Medical Devices Agency (2002). A 3.0 Tesla magnetic resonance scanner (Siemens Trio, Siemens AG, Erlangen, Germany), incorporating a circular polar head and neck coil was used for all scans. Participants were scanned whilst supine with their head in a neutral anatomic position, and wore an adjustable cervical collar to minimise movement of the head and neck, to ensure a reproducible position. During the procedures participants were instructed not to swallow or move, and to rest the tip of their tongue on the back of their teeth during all baseline scans.

2.2.6. Exhaled Nitric Oxide

The inflammatory status of the airway was determined by the analysis of the fractional concentration of exhaled Nitric Oxide (F_ENO) in expired air, using a chemiluminescence analyser (Sievers NOA-280i, Sievers Instruments, Boulder, CO). The measurement of F_ENO has previously been shown to be a valid marker in the diagnosis of asthma (Dupont *et al.*, 2003). The chemiluminescence technique uses the reaction between exhaled NO and ozone to produce an emission in the near-infrared region of the spectrum. The emission is detected by a photomultiplier tube that produces a signal that has a direct relationship with the

NO concentration in the expired air. Measurement of F_ENO was carried out according to current recommendations (ATS/ERS, 2005). Calibration was undertaken prior to all measurements using a calibration gas consisting of NO in Nitrogen at a concentration of 90 ppm. An assumption of linearity was made with human levels of NO that are typically found in the ppb range.

Measurements were performed in a seated position facing a laptop running data acquisition software (Labview, National Instruments, Austin, TX, USA). A nose clip was not used to ensure that nasal NO did not accumulate and contaminate the exhaled sample. Participants were instructed to perform a gradual inhalation to TLC and to exhale immediately. A constant expiratory flow rate of 50 $mL \cdot s^{-1}$ was achieved by expiring against a fixed expiratory resistance and biofeedback of the pressure signal displayed on the laptop. The designated software analysed the F_ENO profile and identified a plateau that conformed to ATS/ERS recommendations. A minimum of three exhalations, at least 1 min apart, with F_ENO values within 10% of the lowest value, were performed to ensure reproducibility. The duration of each exhalation was > 6 s, which corresponded to an exhaled volume of approximately 0.3 L. This allowed the airway compartment to be washed out, increasing the likelihood that a reasonable plateau would be achieved.

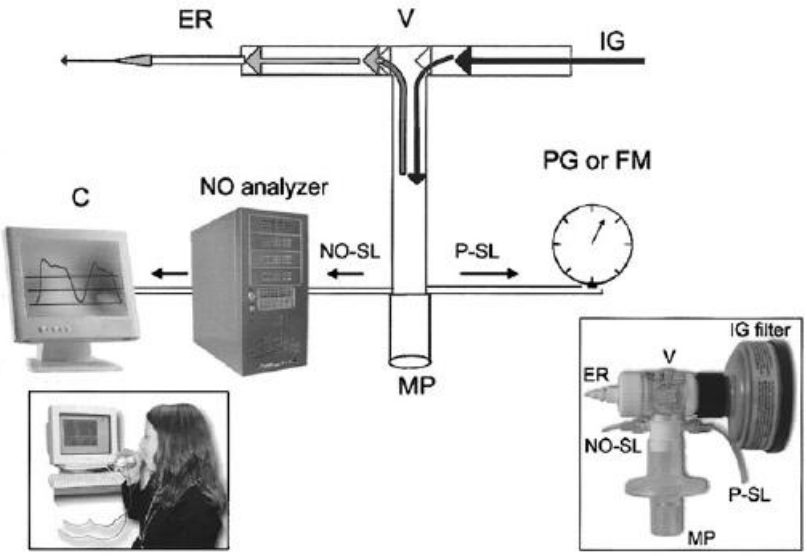

Figure 2.4. Configuration of the breathing circuit used in the restricted-breath technique. *Right bottom insert* shows the restricted-breath configuration that was used in this thesis. C = computer; ER = expiratory resistance; FM = flow meter; IG = inspired gas; MP = mouthpiece; NO-SL = nitric oxide sampling line; PG = pressure gauge; P-SL = pressure sampling line; V = three-way valve. (from ATS/ERS, 2005).

2.2.7. Measurement of Respiratory System Resistance

Respiratory system impedance (Z_{rs}) and its two components, reactance (X_{rs}), and resistance (R_{rs}) were measured using the forced oscillation technique (FOT; Quark i2m, Cosmed, Rome, Italy) in accordance with European Respiratory Society recommendations (Oostveen *et al.*, 2003). The short term and between day coefficient of variation (CV) for the FOT has been reported as 8.3 and 10.0%, respectively (van den Elshout *et al.*, 1990). A low amplitude pseudorandom multi-frequency pressure oscillation (4 – 48 Hz) was applied at the mouth during quiet tidal breathing at functional residual capacity. Baseline measurement was preceded by 5 minutes of quiet tidal breathing during which participants refrained from taking deep breaths or 'sighs', as it is known that lung volume history can influence R_{rs} (Jensen *et al.*, 2001). Volume history was monitored by asking

participants to breathe quietly on a mouthpiece connected to the electronic spirometer described in section 2.2.2. Tidal volume loops were visually inspected in real time on the interfaced PC and any evidence of a deep inhalation (DI) during this period resulted in the 5 min period restarting. Only when participants had breathed for 5 min in the absence of DI were the baseline measurements of R_{rs} made.

Measurements were made whilst sitting, wearing a nose clip, and with the head in a neutral position. Participants breathed on a mouthpiece at FRC, whilst supporting the cheeks and floor of the mouth with both hands to minimise upper airway shunt due to compliance of these structures. The mean of three technically acceptable measurements was recorded. Measurements that showed evidence of artefact on the flow signal were discarded. Prior to each testing session the equipment was calibrated using a calibration cylinder of known resistance in accordance with the manufacturer's guidelines.

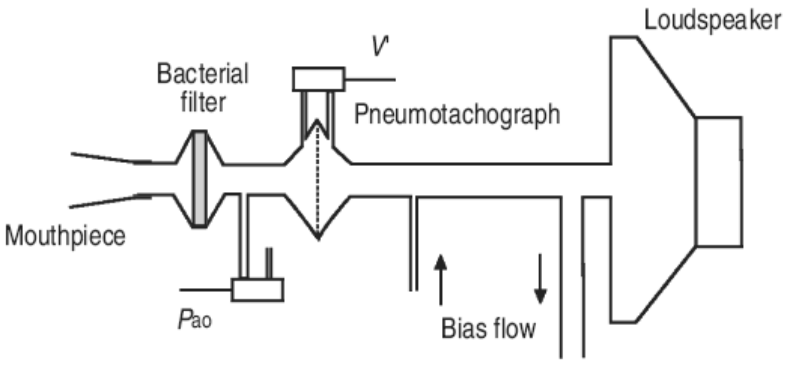

Figure 2.5. Schematic arrangement of the forced oscillatory respiratory impedance measurement. *P*ao: airway opening pressure; *V*': airflow. (from Oostveen *et al.*, 2003).

2.2.8. Incremental Threshold Loading Test

Breathing effort was assessed while participants breathed through a modified version of the pressure-threshold device described in section 2.2.4. Participants breathed against 4 progressive calibrated loads (inspiratory mouth pressures of 10, 20, 30 and 40 cmH_2O) using a self-paced breathing pattern. After breathing for 1 min at each load participants rated the sensation of breathing effort (dyspnoea) using the Borg CR-10 scale (Borg, 1982; see Appendix A-4). The scale is a 12 point scale of verbal descriptors that produces data that grows with an exponent of 1.6, similar to the psychophysical properties known for muscular effort (Noble & Robertson, 1996). The verbal descriptors are anchored to a range of numbers including, 0 (nothing at all), 0.5 (extremely weak), and 10 (extremely strong). The descriptor for 'maximal' is outside of the scale with participants able to assign any number greater than 10 to define this level of effort. The appropriateness of this scale to measure respiratory sensations in people with asthma has previously been demonstrated (Burdon *et al.*, 1982).

Prior to testing all participants read a set of instructions that explained what perceived exertion was and how to use the scale (Appendix A-4). Specifically, the scale was 'anchored' across the perceptual range and participants were advised that there were no right or wrong answers.

CHAPTER THREE

INCREASED ACTIVATION OF LINGUAL MUSCLES IN RESPONSE TO ACUTE INSPIRATORY PRESSURE-THRESHOLD LOADING IN HEALTHY HUMAN BEINGS: AN MRI STUDY

[Note: this chapter has been adapted and published in Respiratory Physiology and Neurobiology]

3.1. INTRODUCTION

Contraction of the thoracic inspiratory muscles produces negative pressures within the intra- and extra-thoracic airway that brings about inspiratory flow. To prevent the natural tendency of the upper airway to collapse during inhalation, the pharyngeal muscles are activated prior to the onset of neural activity to the thoracic inspiratory muscles (Strohl *et al.*, 1980). According to the "balance of pressures" concept, upper airway occlusion occurs when the positive dilating pressure from the upper airway musculature is unable to resist the negative intraluminal pressure caused by inspiratory effort (Brouillette & Thach, 1979). Another approach to understanding airway collapse has been to consider the airway as a collapsible tube. According to this model, the intrinsic properties of the pharyngeal wall determine the collapsibility of the airway (Isono *et al.*, 1997). Contraction of the upper airway dilator muscles produces an adaptive dilating force that stabilises the upper airway and opposes the forces that promote airway collapse (Series, 2002). Consequently, any alteration in the function of the upper airway musculature would interact with airway stability to determine upper airway collapsibility (Series, 2002).

The strength and endurance characteristics of healthy upper airway muscles (genioglossus; GG) during flow resistive loading is such that fatigue precedes that of the thoracic inspiratory muscles (Scardella *et al.*, 1993). In addition, decreased upper airway muscle tone during sleep is a characteristic that may account for increased susceptibility to airway collapse in patients with obstructive sleep apnoea syndrome (OSAS; Oliven *et al.*, 2001). It is known that electromyographic

(EMG) activity of GG is increased during inspiratory flow resistive loading (Malhotra *et al.*, 2000; Pillar *et al.*, 2001), as well as when a negative pressure is applied externally to the upper airway (Aronson *et al.*, 1989; Horner *et al.*, 1991b; Pillar *et al.*, 2001). Thus, it would appear that the muscles of the upper airway are able to be specifically activated in a way that may make them suitable candidates for chronic exercise training.

Pressure-threshold inspiratory muscle training (IMT) is a method of applying a quantifiable external load to the inspiratory muscles. When applied daily over a period of up to 6 weeks, IMT has been shown to improve the function of the thoracic inspiratory muscles (Romer & McConnell, 2003) and to stimulate adaptive changes, including an increase in the percentage of fatigue resistant Type I fibres and an increase in the size of Type II fibres (Ramirez-Sarmiento *et al.*, 2002). If the skeletal muscles regulating the upper airway are also activated during IMT a clear rationale would exist to use this method to condition the upper airway muscles.

Consequently, the aim of this study was to measure the nuclear magnetic resonance (MR) transverse relaxation time (T_2) of muscle water (Fleckenstein *et al.*, 1988; Patten *et al.*, 2003) to determine whether the GG and geniohyoid (GH) muscles are activated in response to an acute bout of pressure-threshold IMT. These muscles were chosen due to their putative role in maintaining upper airway patency (Series, 2002) and because they can be identified clearly on MR images (Ryan *et al.*, 1991; Schotland *et al.*, 1996).

3.2. METHODS

3.2.1. Participants

Eleven healthy participants volunteered for this study. The local Research Ethics Committee approved all experimental procedures (see appendix A-1) and each participant provided written informed consent. All of the participants had pulmonary function within normal limits, as inferred from maximum flow-volume loops. Descriptive characteristics of the participants are shown in Table 3.1.

Table 3.1. Descriptive characteristics of the participants

Parameter	Baseline
n (male/female)	8/3
Age (y)	29 ± 7
Stature (m)	1.74 ± 0.09
Body mass (kg)	72.5 ± 14.5
FEV_1 (L)	4.0 ± 0.8
FVC (L)	5.1 ± 0.9
FEV_1/FVC (%)	82.0 ± 4.7
FEF_{25-75} ($L \cdot s^{-1}$)	4.1 ± 1.0
PIF ($L \cdot s^{-1}$)	8.0 ± 1.6
MIP at RV supine (cmH_2O)	112 ± 28

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25-75}, forced expiratory flow between 25 and 75% of FVC; PIF, peak inspiratory flow; MIP, maximum inspiratory mouth pressure at residual volume (RV); MEP, maximum expiratory mouth pressure. All pulmonary function values were within normal limits (Quanjer *et al.*, 1993). Values are mean ± SD.

3.2.2. Procedures

Protocol

Participants underwent T_2-weighted MR imaging (MRI) scans of the upper airway: two baseline scans separated by 5 minutes (control) and one immediately after an acute bout of pressure-threshold IMT. A commercially available training device (POWERbreathe®, HaB Ltd, Southam, UK) adapted for MRI use was

connected to the subject via a flanged mouthpiece and remained in place throughout all of the scans. For the baseline scans the participants breathed quietly via the nose. For the IMT bout, the participants occluded their nose with a clip and performed dynamic inspiratory efforts at 60% of MIP to task failure (~ 5 min). Participants were instructed to initiate the inspiratory efforts from residual volume (RV) and to continue up to the lung volume where the inspiratory muscle force output for the given load limited further excursion of the thorax. Because of the increased tidal volume, a decreased breathing frequency was adopted to avoid hyperventilation and the consequent hypocapnia. At task failure, the subjects removed the nose clip and returned to quite nasal breathing. In 7 of the subjects (4 males), the time course of T_2 change was determined by repeating the scans at 5 and 10 min after IMT. Each of the scans took < 5 min to complete and was acquired in the axial plane from the base of the epiglottis to the level of the hard palate at sequentially increasing spin echo times (Table 3.2).

Table 3.2. Magnetic resonance imaging parameters

Parameter	Axial
Repetition time (ms)	2000
Echo time (ms)	34, 68, 85, 120
Acquisition time (s)	280
Acquisition matrix (mm)	256 × 192
Field of view (mm)	210
Slice thickness (mm)	5
Slice separation (mm)	0

Pulmonary function

Maximal flow-volume loops were assessed using an online spirometer (Oxycon Pro, Jaeger, Hoechberg, Germany) 24 hrs prior to the main experiment. All

measurements were performed and interpreted according to European Respiratory Society/American Thoracic Society (ATS/ERS) guidelines (Miller *et al.*, 2005; Pellegrino *et al.*, 2005). Procedural details are described in section 2.2.2.

Respiratory muscle function

Inspiratory muscle strength was assessed to set the individual inspiratory load used in the acute bout of loaded breathing. Participants lay supine whilst maximum inspiratory mouth pressure (MIP) was determined from RV. Maximal mouth pressures were measured using a digital mouth pressure meter (Micro RPM, Micro Medical Ltd, Rochester, Kent, UK) and were defined as the highest pressure averaged over one second from three manoeuvres that varied by less than 10% (ATS/ERS, 2002). Procedural details are described in section 2.2.3.

Magnetic resonance imaging

Measurements were performed using a 3.0 Tesla MR scanner (Siemens Trio, Siemens AG, Erlangen, Germany) with a circular polar head/neck coil. Since neck position alters upper airway anatomy (Jan *et al.*, 1994) subjects were aligned in the Frankfort plane, perpendicular to the scanner table, and secured in this position with foam pads placed between their head and each side of the head/neck coil. In addition, the subjects wore an adjustable cervical collar to further minimise movement of the head and neck and to ensure a reproducible position. The subjects were instructed to rest the tip of their tongue on the back of their teeth during all baseline scans and not to swallow or move during the procedures. Procedural details are described in section 2.2.5.

Analysis of images

Analysis of MR images was similar to that described previously (Schotland *et al.*, 1999). Briefly, the images were analysed by the same investigator using National Institutes of Health ImageJ software (Rasband, 2005). As spin echo time increases the image intensity decreases for each pixel, and this decrease follows an exponential decay curve that is used to generate a T_2 relaxation time for every pixel. These individual pixel T_2 values were used to generate a T_2 map, which is essentially a visual representation of the T_2 values for all the pixels in the image (Figure 3.1). The slice containing the largest surface area for the muscle of interest (GH or GH) was initially selected from an anatomic guide image. The anatomic guide was the first spin echo image (echo time = 34 ms) in the series used to make up the T_2 map. The region of interest was initially selected with a cursor on the anatomic guide image and then automatically transposed onto the T_2 map. The median T_2 value for the selected muscle was calculated from the T_2 map. Within-occasion reproducibility (coefficient of variation, CV) of the resting baseline T_2 values was 2.0% for GH and 2.3% for GG.

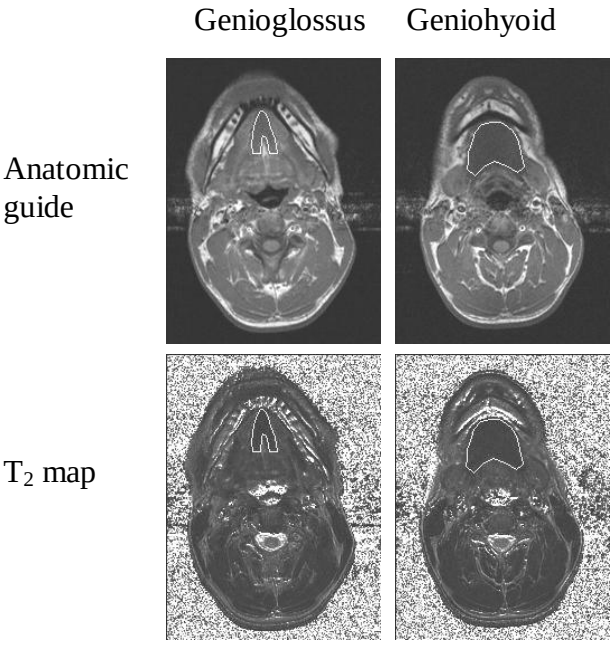

Figure 3.1. Anatomic guide and corresponding T_2 map of the geniohyoid and genioglossus in a representative subject. The guide image was the first spin echo image (echo time = 34 ms) of the series acquired to make up each T_2 map. The region of interest was initially selected using a cursor from the anatomic guide image and then transposed onto the T_2 map.

3.2.3. Statistical Analyses

Repeated measures ANOVA was used to examine for group mean differences in T_2 values across "time" (pre 1, pre 2, 0 min post, 5 min post, 10 min post) for GG and GH. Mauchly's sphericity test was used to check homogeneity of covariance and violations of this assumption were corrected using the Greenhouse-Geisser adjustment. Following significant main effects, planned pairwise comparisons were made using the Bonferroni method. Statistical significance was set at $P < 0.05$. Results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS v13.0 for Windows (SPSS Inc., Chicago IL, USA).

3.3. RESULTS

The T_2 values were not different between the two baseline scans for either GH or GG (Figure 3.2). Immediately after the acute bout of IMT, T_2 values were elevated above baseline for GG ($P < 0.001$) and GH ($P < 0.001$) (Figure 3.2). These increases in T_2 represented 111 ± 6% and 116 ± 9% of baseline for GG and GH, respectively. In the subgroup of 7 subjects, T_2 values immediately after acute IMT were elevated, but did not differ from baseline (pre 1) after 5 and 10 min of recovery (Figure 3.3).

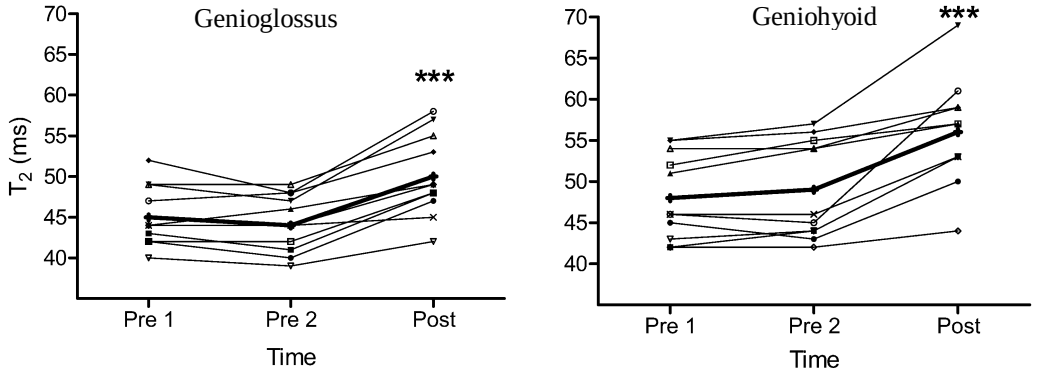

Figure 3.2. Individual and group mean (in bold) pixel T_2 values for genioglossus and geniohyoid before (Pre 1 and Pre 2) and immediately after an acute bout of pressure-threshold IMT (n = 11 subjects). *** $P < 0.001$, significantly different from Pre 1 and Pre 2.

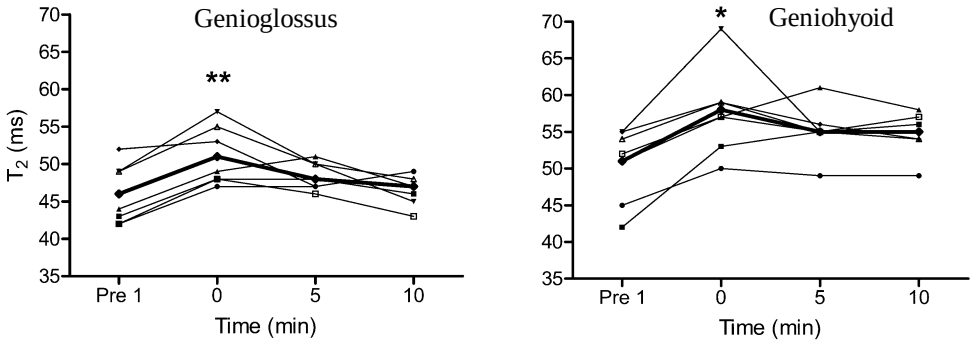

Figure 3.3. Individual and group mean (in bold) pixel T_2 values for genioglossus and geniohyoid before (Pre 1), immediately after, and 5 and 10 min after an acute bout of pressure-threshold IMT (n = 7 subjects). * $P < 0.05$, significantly different from Pre 1; ** $P < 0.01$, significantly different from Pre 1.

3.4. DISCUSSION

3.4.1. Main Finding

The main finding was that selected upper airway dilator muscles (GG and GH) were activated in response to an acute bout of pressure-threshold IMT, as demonstrated by prolongation of T_2 relaxation times.

The finding that IMT increased the activation of specific upper airway dilator muscles is new but in line with the original hypothesis. Current thought holds that activity-induced increases in T_2 result from intracellular acidification in combination with an effect of intracellular water accumulation on muscle protein concentration (Patten *et al.*, 2003). That the largest increase in T_2 occurred within 5 min post-IMT is in agreement with previous studies in limb muscles (Fleckenstein *et al.*, 1988; Fisher *et al.*, 1990) and supports the notion that the increase in T_2 is linked to work-related changes in the metabolic activity of muscle cells. Whatever the mechanism underlying the increase in T_2, there is considerable data indicating that the method provides valuable practical information about muscle use. The magnitude of activity-induced elevations in T_2 is strongly related to limb muscle EMG activity, force and power (Fisher *et al.*, 1990; Adams *et al.*, 1992; Adams *et al.*, 1993; Jenner *et al.*, 1994; Ploutz *et al.*, 1994; Yue *et al.*, 1994). Further, T_2 analysis has been used to identify the location (Fleckenstein *et al.*, 1989; Fleckenstein *et al.*, 1992; Sloniger *et al.*, 1997b; Green & Wilson, 2000) and relative intensity (Fisher *et al.*, 1990; Fleckenstein *et al.*, 1992; Flicker *et al.*, 1993; Conley *et al.*, 1995; Sloniger *et al.*, 1997a, b) of muscle recruitment during various motor tasks.

Although this study is the first to use the T_2 method to investigate the effect of acute IMT on the activation of upper airway dilator muscles, the method has been used in animals and patients with OSAS to demonstrate alterations in the properties of several upper airway muscles, that are compatible with the presence of oedema as a result of muscle overuse (Schotland *et al.*, 1996; Schotland *et al.*, 1999). In addition, the method has been used to show reductions in upper airway mucosal water content in response to chronic nasal continuous positive airway pressure therapy in patients with OSAS (Ryan *et al.*, 1991). In support of these findings, studies using EMG have shown an increased activation of GG in response to inspiratory resistive loading (Malhotra *et al.*, 2000; Pillar *et al.*, 2001) and negative pressure ventilation (Aronson *et al.*, 1989; Horner *et al.*, 1991b; Pillar *et al.*, 2001). Disadvantages of such studies, however, are that EMG can be invasive and limited by the problem of accessing the muscles of interest. Furthermore, measuring GG activity with the use of intramuscular electrodes is problematic given the heterogeneous behaviour of this muscle (Eastwood *et al.*, 2003). In contrast, T_2 analysis has the advantage of allowing objective analysis of intact airway muscles without the use of invasive procedures. In addition, the method is able to detect small T_2 changes, as demonstrated by the relatively low typical errors for the baseline T_2 measurements in both GG and GH (CV ~2.0%). Although this study showed increases in T_2 in both GG and GH, it is likely that additional upper airway dilator muscles were also activated; in particular, the styloglossus, hyoglossus and intrinsic tongue muscles, since these are also innervated by the hypoglossus nerve.

This study has demonstrated that in healthy awake subjects, GG and GH activation was increased in response to an acute bout of pressure-threshold IMT. This finding supports the use of IMT as a potential method to strengthen the upper airway muscles that may be important in preventing airway collapse. Further studies are required to determine the functional consequences of chronic IMT on the upper airway dilator muscles in healthy and patient populations.

CHAPTER FOUR

EFFECT OF SIX WEEKS INSPIRATORY PRESSURE-THRESHOLD LOADING ON THE UPPER AIRWAY RESPONSE TO LOADED BREATHING: AN MRI STUDY

[Note: this chapter has been adapted and published in Respiratory Physiology and Neurobiology]

4.1. INTRODUCTION

In Chapter 3, evidence was presented for increased activation of specific upper airway dilator muscles (genioglossus (GG) and geniohyoid (GH)) after an acute bout of pressure-threshold inspiratory muscle training (IMT). Using magnetic resonance imaging (MRI) to determine the transverse relaxation time of muscle water (T_2) significant increases in T_2, within 5 min of completing an acute bout of IMT, were reported. This finding corroborates previous work that has studied limb muscles (Fleckenstein *et al.*, 1988; Fisher *et al.*, 1990) and supports existing opinion that increases in T_2 are linked to work-related increases in the metabolic activity of muscle cells (Patten *et al.*, 2003).

Previous studies have demonstrated improved functional outcomes after a period of activity that chronically overloads the upper airway dilator muscles. Four weeks of voluntary isocapnic hyperpnoea training was found to reduce the incidence of snoring (Furrer *et al.*, 1998), whilst four months of didgeridoo playing improved sleep-related outcomes in patients with obstructive sleep apnoea syndrome (OSAS; Puhan *et al.*, 2006). In addition, eight weeks of tongue-muscle training by intraoral electrical neurostimulation was found to reduce the incidence of snoring in patients with OSAS (Randerath *et al.*, 2004). More recently Guimaraes *et al.* (2009) demonstrated reduced OSAS severity and symptoms after 3 months of oropharyngeal exercises. Also, a number of case study reports show that pressure-threshold IMT is effective in treating vocal cord dysfunction (Sapienza *et al.*, 1999; Baker *et al.*, 2003a; Baker *et al.*, 2003b; Mathers-Schmidt & Brilla, 2005) and inspiratory stridor (Dickinson *et al.*, 2007), which suggests

that IMT activates the upper airway muscles in a way that enhances their function. Further, a study by Vincent *et al.* (2002) provides more objective evidence in support of the adaptability of the upper airway muscles to a training stimulus. These authors reported that rodents exhibit a fast to slow shift in myosin heavy chain phenotype, and increased oxidative and antioxidant capacity, in the upper airway muscles in response to the hyperpnoea of endurance training. The evidence above, alongside that presented in Chapter 3, supports the notion that improvements in functional outcomes after upper airway training are a direct result of chronic adaptations to the airway dilator muscles and that pressure-threshold IMT provides a suitable means to impose the training stimulus.

Chronic resistance exercise training elicits positive changes in the force, velocity and endurance characteristics of muscle that are dependent on the frequency, intensity and duration of the exercise (Harridge, 2007). In addition, it is known that chronic exercise training increases the passive stiffness of locomotor muscles, independent of increases in either muscle mass or force output (Lindstedt *et al.*, 2002). Thus, if an adequate training stimulus was imposed upon the upper airway dilator muscles, it is reasonable to speculate that there would be a reduced tendency of the upper airway to collapse due to an increase in the active (neural) tone, an increase in the passive (intrinsic) stiffness of the pharyngeal dilators, or both.

In light of these observations this study aimed to determine whether there was a dose-response relationship between the magnitude of inspiratory loading and

subsequent narrowing of the upper airway as assessed by 2D-Flash MRI. In addition, the effect of 6 weeks IMT on this relationship was also examined. It was hypothesised that a dose-response relationship would be present, whereby more negative pressures would result in greater narrowing of the airway. Further, it was predicted that after IMT, airway narrowing would be attenuated at each level of inspiratory loading.

4.2. METHODS

4.2.1. Participants

Nine participants volunteered to take part in this study. The local Research Ethics Committee approved all experimental procedures (see appendix A-1) and each participant provided written informed consent. All of the participants had pulmonary function within normal limits, as inferred from maximum flow-volume loops. Descriptive characteristics of the participants are shown in Table 4.1.

Table 4.1. Descriptive characteristics of the participants

Parameter	Baseline	Post-IMT
n (male/female)	5/4	-
Age (y)	25.2 ± 4.7	-
Stature (m)	1.76 ± 0.11	-
Body mass (kg)	69.0 ± 14.2	-
FEV_1 (L)	4.5 ± 0.8	4.4 ± 0.8
FVC (L)	5.3 ± 0.8	5.2 ± 0.9
FEV_1/FVC (%)	84.7 ± 2.7	85.0 ± 2.7
FEF_{25-75} ($L \cdot s^{-1}$)	4.7 ± 1.2	4.6 ± 1.3
PIF ($L \cdot s^{-1}$)	8.2 ± 2.7	8.4 ± 2.0
MIP at RV standing (cmH_2O)	96 ± 18	126 ± 24***
MIP at FRC supine (cmH_2O)	55 ± 10	72 ± 14***
MEP (cmH_2O)	156 ± 46	155 ± 45

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25-75}, forced expiratory flow between 25 and 75% of FVC; PIF, peak inspiratory flow; MIP, maximum inspiratory mouth pressure at either residual volume (RV) or functional residual capacity (FRC); MEP, maximum expiratory mouth pressure. All pulmonary function values were within normal limits (Quanjer *et al.*, 1993). *** $p < 0.001$, significantly different from baseline. Values are mean ± SD.

4.2.2. Procedures

Protocol

Participants underwent two MRI scans of the upper airway separated by 6 weeks of pressure-threshold IMT. During these scans the participants inspired against flow resistive loads corresponding to 10, 30 and 50% of their pre-training

maximum inspiratory mouth pressure (MIP). After a localising scan, images were acquired in the axial and sagittal plane using a 2D-Flash sequence (Table 4.2). All images were taken from the base of the epiglottis to the level of the hard palate.

Table 4.2. Magnetic resonance imaging parameters

Parameter	Sagittal	Axial
Repetition time (ms)	5.6	5.6
Echo time (ms)	2.29	2.29
Acquisition time (s)	7	12
Field of view (mm)	200	200
Slice thickness (mm)	5	4
Effective slice thickness (mm)	6	4

The order of the resistive loads was randomised and counterbalanced. Four manoeuvres were performed at each percentage of MIP - two while scanning in the sagittal plane and two in the axial plane. In addition, four resting scans (two sagittal and two axial) were performed both before and after the inspiratory manoeuvres. All of the inspiratory manoeuvres were performed from functional residual capacity (FRC), as assessed via inductance pneumography. Participants performed the inspiratory manoeuvres via a semi-occluded mouthpiece, which was attached to a length of polyethylene tubing identical to that used for the determination of the inspiratory loads (see respiratory muscle function below). The tubing was connected to an electro-manometer (Mercury Electronics, Glasgow, UK) which, in turn, was interfaced to a laptop computer running a target-pressure protocol programme (Labview, National Instruments, Austin, TX, USA). The computer screen image was visible to the participants via a projector link that was displayed on the screen above their head in the scanner. A graphical representation of the required and actual pressures was displayed and when these

two pressures were in agreement scanning commenced. Participants were instructed to sustain the inspiratory manoeuvre until the scan finished, which was 12 and 7 s for the axial and sagittal slices, respectively. After each scan, the target and actual pressure data were saved to an Excel spreadsheet.

Lung function

Maximum flow-volume loops were assessed at baseline and after 6 wk of IMT using an online spirometer (Oxycon Pro, Jaeger, Hoechberg, Germany). All measurements were performed and interpreted according to European Respiratory Society/American Thoracic Society (ATS/ERS) guidelines (Miller *et al.*, 2005; Pellegrino *et al.*, 2005). Procedural details are described in section 2.2.2.

Respiratory muscle function

Inspiratory muscle strength was assessed to set the individual inspiratory loads for each participant and to monitor the effectiveness of the IMT. Expiratory muscle strength was assessed to control for learning effects. MIP was assessed from residual volume (RV) whilst standing to monitor changes in inspiratory muscle strength due to IMT, and from FRC whilst supine in order to set the acute inspiratory loads. Maximum expiratory mouth pressure (MEP) was determined from total lung capacity (TLC). Measurements from FRC were made using an arrangement similar to that of the MRI protocol described previously. Specifically, participants performed a maximal inspiratory manoeuvre via a semi-occluded mouthpiece attached to a length of polyethylene tubing that was connected at its distal end to the pressure meter. The mouthpiece was linked to a

wedge spirometer (Spirotrac II, Vitalograph Ltd, Buckingham, UK) via a two-way non-re-breathing valve arrangement, which ensured that all inspiratory manoeuvres were performed from FRC. All pressures were measured using a digital mouth pressure meter (Micro RPM, Micro Medical Ltd, Rochester, Kent, UK). MIP and MEP were defined as the highest pressure averaged over one second from three manoeuvres that varied by less than 10% (ATS/ERS, 2002). Procedural details are described in section 2.2.3.

Magnetic resonance imaging

Measurements were performed using a 3.0 Tesla MR scanner (Siemens Trio, Siemens AG, Erlangen, Germany) with a circular polar head/neck coil. Since neck position alters upper airway anatomy (Jan *et al.*, 1994) participants were aligned in the Frankfort plane, perpendicular to the scanner table, and secured in this position with foam pads placed between their head and each side of the head/neck coil. In addition, the participants wore an adjustable cervical collar to further minimise movement of the head and neck and to ensure a reproducible position. The participants were instructed to rest the tip of their tongue on the back of their teeth during all baseline scans and not to swallow or move during the procedures. Procedural details are described in section 2.2.5.

Analysis of images

Images captured at rest and during the inspiratory manoeuvres were analysed using National Institutes of Health ImageJ software (Rasband, 2005) with the investigator blinded to whether the data were from pre- or post-IMT. These

measurements were averaged to give a mean value for each percentage of MIP for every subject. Axial images were analysed for: 1) airway cross-sectional area (mm^2); 2) lateral diameter (mm); and 3) anteroposterior diameter (mm). Measurement of the axial images (pre- and post-IMT) was taken from the slice that showed the greatest reduction in cross-sectional area (CSA) at 50% MIP (pre-IMT). The computer software applied an automatic threshold to the image that enabled the airway to be differentiated from the surrounding tissue. An automatic measurement tool within the software was used to evaluate the various regions of interest. Sagittal images were analysed for: 1) anteroposterior diameter of the nasopharynx (mm); 2) anteroposterior diameter of the oropharynx (mm); and 3) anteroposterior diameter of the laryngopharynx (mm). Sagittal images were partitioned into three planes similar to those described previously (Stuck *et al.*, 2002). Three parallel lines were drawn at the level of the nasopharynx, oropharynx and laryngopharynx using the anterior arch of the atlas and vertebra C3 and C4 as anatomic markers, respectively. In the resting subject, the cross-sectional area of the tongue and associated muscles (mm^2); the distance between the anterior surface of the mandible to the base of the tongue (mm); and the retrolingual airway space between the nasopharynx and laryngopharynx planes (mm^2) were also analysed (Figure 4.1) as described previously (Stuck *et al.*, 2002). The cross-sectional area of the tongue and the retrolingual airway space were calculated automatically after circumscribing the regions of interest using the image analysis software. The mean CV for two additional participants over four trials each separated by 2 weeks are shown in Table 4.3.

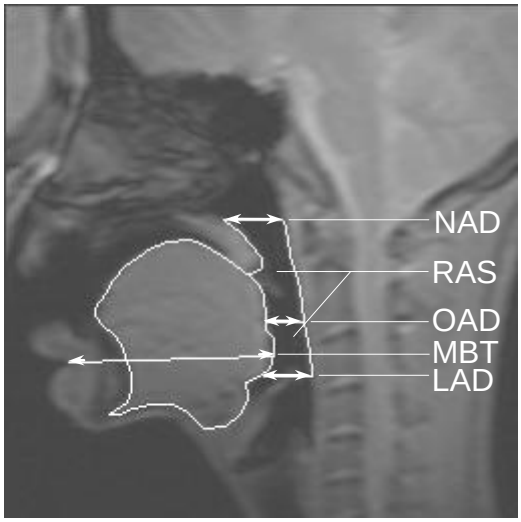

Figure 4.1. Parameters analysed in the sagittal plane. NAD, nasopharynx anteroposterior diameter; RAS, retrolingual airway space; OAD, oropharynx anteroposterior diameter; MBT, mandible to the base of the tongue; LAD, laryngopharynx anteroposterior diameter.

Table 4.3. Within-subject, between occasion reliability (coefficient of variation, %) for each parameter across each of the inspiratory loads in the sagittal and axial planes.

Parameter	Rest 1	10% MIP	30% MIP	50% MIP	Rest 2
Sagittal plane					
Nasopharynx anteroposterior diameter	13.0	0.0	0.0	0.0	19.4
Oropharynx anteroposterior diameter	19.3	17.8	17.1	22.8	22.8
Laryngopharynx anteroposterior diameter	16.9	10.2	12.6	16.5	12.5
Tongue cross-sectional area	7.8	-	-	-	-
Mandible to base of tongue	3.0	-	-	-	-
Retrolingual airway space	16.3	-	-	-	-
Axial plane					
Airway cross-sectional area	27.4	18.8	18.8	11.0	27.6
Lateral diameter	11.9	8.6	9.2	10.0	10.0
Anteroposterior diameter	16.6	10.6	13.2	9.8	16.9

Note: Data are mean for 2 participants. Each subject underwent 4 separate scans each separated by 2 weeks. The posterior airway space at the nasopharynx became occluded during loaded breathing; therefore, all measurements at this plane equated to zero.

Inspiratory muscle training

Participants performed 30 inspiratory efforts twice-daily for 6 weeks using a commercially available training device (POWERbreathe®, HaB Ltd, Southam, UK; Caine & McConnell, 2000). Participants were instructed to inspire fully and with maximal effort from RV to TLC. The inspiratory load was initially set to 50% MIP - a load that is known to improve thoracic inspiratory muscle function (Romer & McConnell, 2003). The participants were instructed to increase the load periodically to a level that would only just allow them to complete the 30 efforts (~ 60% MIP). Six weeks of IMT was used because it has been shown that this duration produces a physiological plateau in strength and power development of the thoracic inspiratory muscles (Romer & McConnell, 2003). Training diaries were used to monitor IMT compliance, and the participants were instructed to cease training 48 h before the post-IMT scans. Procedural details are described in section 2.2.4.

4.2.3. Statistical Analyses

Repeated measures ANOVA was used to examine for group mean differences in airway measurements between "inspiratory load" (rest 1, 10% MIP, 30% MIP, 50% MIP, rest 2) and "trial" (pre-IMT, post-IMT). Mauchly's sphericity test was used to check homogeneity of covariance and violations of this assumption were corrected using the Greenhouse-Geisser adjustment. Following significant main effects, planned pairwise comparisons were made using the Bonferroni method. Pre- vs. post-IMT differences in pulmonary and respiratory muscle function measurements were examined using paired *t*-tests. Within-subject, between

occasion reliability was determined using the coefficient of variation. Statistical significance was set at $P < 0.05$. Results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS v13.0 for Windows (SPSS Inc., Chicago IL, USA).

4.3. RESULTS

4.3.1. Acute Responses to Inspiratory Resistive Loading

Actual mouth pressure did not differ from target pressure across either inspiratory load or trial. The acute response of the upper airway to inspiratory resistive loading in the axial plane in a typical subject is shown in Figure 4.2. Group mean and individual subject data are presented in Figure 4.3 and Table 4.4, respectively.

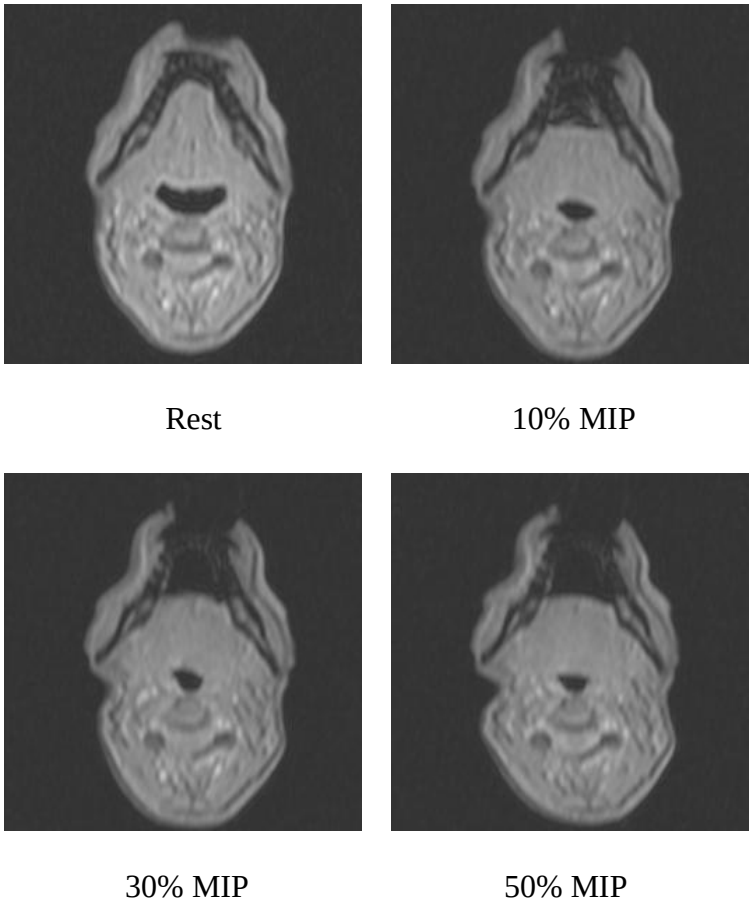

Figure 4.2. Airway cross sectional area at rest and during loaded breathing at 10, 30 and 50% maximum inspiratory mouth pressure (MIP) in a representative subject. Measurements were made at the point of most narrowing at 50% MIP. Note the extent of airway narrowing at 10% MIP with no further narrowing at higher pressures. Most of the narrowing occurred in the lateral diameter.

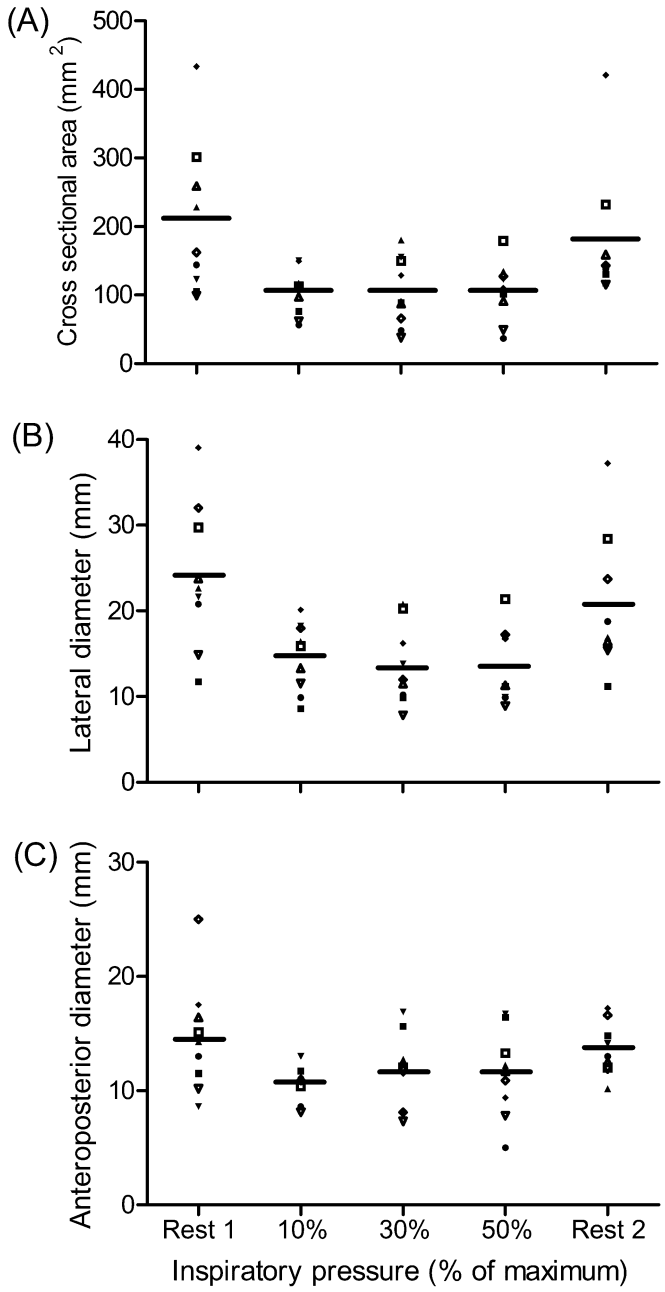

Figure 4.3. Effect of inspiratory loading on cross-sectional area (panel A), lateral diameter (panel B) and anteroposterior diameter (panel C). Individual and group mean data (bars). *n* = 9 participants. See Table 4.4 for location of group mean differences.

Table 4.4. Upper airway response to acute inspiratory resistive loading at the site of most narrowing (axial plane), and at the naso, oro and laryngopharynx (sagittal plane).

Parameter	Rest 1			10% MIP			30% MIP			50% MIP			Rest 2		
	Pre-IMT	Post-IMT	Mean	Pre-IMT	Post-IMT	Mean	Pre-IMT	Post-IMT	Mean	Pre-IMT	Post-IMT	Mean	Pre-IMT	Post-IMT	Mean
Axial plane															
Airway CSA (mm^2)															
Mean	212	216	214 $_{a}$	103	111	107 $_{b}$	105	98	102 $_{b}$	106	120	113 $_{b}$	182	185	184 $_{a}$
SD	117	113	115	34	44	39	51	71	61	44	54	49	104	109	107
Lateral diameter (mm)															
Mean	24.0	22.7	23.4 $_{a}$	14.7	16.1	15.4 $_{b}$	13.6	12.9	13.3 $_{b}$	13.7	15.2	14.5 $_{b}$	20.4	20.9	20.7 $_{c}$
SD	8.5	7.3	7.9	4.0	5.4	4.7	4.6	5.5	5.1	4.4	5.1	4.8	8.1	7.6	7.9
Anteroposterior diameter (mm)															
Mean	14.6	14.1	14.4 $_{a}$	10.6	11.1	10.9 $_{a}$	11.6	10.6	11.1 $_{a}$	11.5	12.1	11.8 $_{a}$	13.6	13.5	13.6 $_{a}$
SD	4.9	2.9	3.9	1.5	3.2	2.4	3.3	3.5	3.4	3.8	4.1	3.9	2.3	2.8	2.6
Sagittal plane															
Nasopharynx (mm)															
Mean	12.2	12.9	12.6 $_{a}$	0.0	0.0	0.0 $_{b}$	0.0	0.0	0.0 $_{b}$	0.0	0.0	0.0 $_{b}$	12.0	11.6	11.8 $_{a}$
SD	3.8	3.9	3.7										4.7	3.7	4.2
Oropharynx (mm)															
Mean	10.5	11.2	10.9 $_{a}$	9.2	9.5	9.4 $_{a}$	9.4	9.8	9.6 $_{a}$	10.2	10.2	10.2 $_{a}$	10.5	11.5	10.8 $_{a}$
SD	1.7	2.7	2.2	2.3	2.4	2.4	2.9	3.0	3.0	3.5	2.8	3.2	1.7	3.5	2.6
Laryngopharynx (mm)															
Mean	14.6	15.0	14.8 $_{a}$	12.0	12.8	12.4 $_{ab}$	9.6	10.3	10.0 $_{c}$	10.0	10.2	10.1 $_{c}$	13.1	13.3	13.2 $_{bc}$
SD	4.7	4.4	4.6	4.8	4.2	4.5	3.8	5.3	4.6	3.1	3.9	3.5	4.8	3.9	4.4

Note: Values pre-IMT vs. post-IMT were not significantly different ($P > 0.05$). Means in the same row that do not share subscripts differ at $P < 0.05$

In the axial plane, there was a main effect of inspiratory load on upper airway cross-sectional area (P = 0.025) and lateral diameter (P = 0.003). Parameter values were both reduced at 10% MIP compared to rest (P = 0.012 and 0.001, respectively), but no further reductions were found at 30 and 50% MIP. There was no effect of inspiratory load on anteroposterior diameter (P > 0.05).

In the mid-sagittal plane, there was a main effect of inspiratory load on airway diameter at the nasopharynx (P < 0.001) and at the laryngopharynx (P = 0.031). During all loaded inhalations the airway at the nasopharynx was occluded due to movement of the soft palate and uvula against the posterior airway wall. At 30 and 50% MIP, the airway diameter at the laryngopharynx was less versus rest 1 (P = 0.02 and 0.02, respectively). There was no effect of inspiratory load on airway diameter at the oropharynx (P = 0.28).

4.3.2. Effect of Inspiratory Muscle Training

Participants completed 79 of the 84 IMT sessions (94% adherence). There was no effect of IMT upon pulmonary function (Table 4.1). MIP increased (96 ± 18 vs. 126 ± 24 cmH_2O, P < 0.001; 33 ± 14%), but MEP did not change (156 ± 46 vs. 155 ± 45 cmH_2O; P = 0.97). There was no effect of IMT upon the upper airway responses to inspiratory loading in either the axial plane or the sagittal plane (Table 4.4). In addition, there was no effect of IMT on resting measurements of retrolingual airway space (530 ± 19 vs. 563 ± 6 mm^2; P = 0.24), tongue cross-sectional area (2833 ± 376 vs. 2936 ± 498 mm^2; P = 0.22) or distance between the

anterior surface of the mandible to the base of the tongue (73.9 ± 5.7 vs. 74.0 ± 5.7 mm; $P = 0.85$).

4.4. DISCUSSION

4.4.1. Main Findings

The main finding was a substantial reduction in upper airway cross-sectional area at relatively low inspiratory resistive loads (10% MIP) that did not worsen at higher loads (30 and 50% MIP). The majority of airway narrowing in the axial plane occurred in the lateral diameter of the airway, with narrowing in the anteroposterior plane occurring at the laryngopharynx at higher inspiratory loads (≥ 30% MIP). Further, under the conditions of the present study it was not possible to detect any effect of 6 wk pressure-threshold IMT upon upper airway morphology at rest, or the extent of upper airway narrowing during inspiratory resistive loading.

4.4.2. Acute Responses to Inspiratory Resistive Loading

Airway cross-sectional area in the axial plane was reduced by almost one-half at 10% MIP with no further reductions at 30 or 50% MIP. The majority of the reduction in cross-sectional area was due to mobility of the lateral pharyngeal walls, as evidenced by a significant reduction in lateral airway diameter. In the sagittal plane, complete occlusion of the nasopharynx occurred at 10% MIP due to movement of the soft palate and uvula against the posterior pharyngeal wall. At 30 and 50% MIP, the anteroposterior airway diameter at the level of the laryngopharynx decreased by about 30%.

Changes in upper airway dimensions in response to actively generated negative pressure in healthy awake subjects have been reported by some (Rodenstein *et al.*,

1983), but not all previous studies (Wheatley *et al.*, 1991). Rodenstein *et al*. (1983) used cineradiography to show that the airway diameter during panting against a closed shutter changed more in the lateral plane (48 and 82% for mouth pressures swings of ~ 12 and 28 cmH_2O, respectively) than in the anteroposterior plane (21 and 52%). Moreover, both diameter changes were correlated with the corresponding pressure swings (r = 0.50 to 0.57, $P < 0.10$), such that a greater pressure swing resulted in a larger change in diameter. Unfortunately, the authors did not report whether most of the dimension change occurred during the positive or negative pressure swing. In agreement with our findings at 10% MIP (–9.6 cmH_2O), Wheatley *et al*. (1991) showed using X-ray fluoroscopy that the anteroposterior airway diameter at the level of the 3rd and 4th cervical vertebrae did not change during the active generation of negative intraluminal pressures up to 15 cmH_2O. In contrast to our findings, however, these authors did not find significant changes in the lateral diameter of the airway at the level of the C4 vertebra (Wheatley *et al.*, 1991). The reason for the contrasting findings is unclear but may be due, in part, to differences in the method of pressure application (i.e., a gradual ramp versus the rapid and sustained increase in pressure in the present study). These findings, in combination with those from the present study, suggest that lateral narrowing occurs at low transmural pressures without a dose-response relationship. Further, anteroposterior narrowing occurs at higher pressures, but does not exhibit a dose-response, at least not up to 50% of MIP.

4.4.3. Effect of Inspiratory Muscle Training

Under the conditions of the present study no chronic changes in either the morphology of the upper airway, or its collapsibility during inspiratory resistive loading were detected. Although it was shown in Chapter 3 that activity of GG and GH was elevated in response to a single bout of IMT, it is possible that IMT did not present a strong enough stimulus to elicit a training response. However, recent evidence of a strong relationship between upper airway muscle strength and inspiratory muscle strength (Shepherd *et al.*, 2006), coupled with the finding of an increase in the strength of these inspiratory muscles with IMT, suggests that the upper airway dilator muscles were subject to a training stimulus.

All reasonable precautions were taken to control for errors in the measurements. The same investigator analysed all of the images because intra-investigator variability is less than inter-investigator variability (Stuck *et al.*, 2002). The images were carefully matched before analysis and the investigator was blinded to whether the scans were taken before or after IMT. As pharyngeal cross-sectional area varies with lung volume, increasing as total lung capacity is attained, some error may have been introduced due to an effect of operating lung volume on upper airway dimensions (Brown *et al.*, 1986). Such an effect was unlikely to have influenced our pre- versus post-IMT comparisons because each of the inspiratory loading manoeuvres was initiated from the same lung volume (FRC) and absolute pressures (and hence lung volumes) did not differ pre- versus post-IMT. Furthermore, lung volume changes during resting tidal breathing were unlikely to have affected upper airway dimensions because the measurements

were averaged across multiple images and over several breaths. Thus, the extent of the variation that can be attributed to measurement technique was considered to be minimal.

The within-subject, between occasion, reliability coefficients for resting measurements in the sagittal plane for two participants each scanned on four different occasions (CV = 3 to 19%) were similar to those reported by Stuck *et al*. (2002; CV = 3 to 14%). In line with these authors, it was shown that the highest levels of variability occurred with the measurement of the anteroposterior diameters (CV = 13 to 17%) and the retrolingual airway space (CV = 16%). The measurements that were taken in the axial plane also showed differing degrees of variability with the most reliable measure being the lateral airway diameter (CV = 12%). Of all the parameters measured during inspiratory loading, the lateral airway diameter was the most stable over time (CV $\leq$ 10%). With nine participants, if IMT had attenuated lateral airway narrowing by 30% during the 10% MIP manoeuvre, the reliability data suggest that this would be detectable 80% of the time. In order to detect a 10% effect (~1.5 mm) this study would have required 70 participants, which is neither feasible logistically, nor realistic financially. Since measurement error was likely to be negligible (see previous paragraph), it is reasonable to attribute these relatively high levels of variability to inherent biological variation in the structures of the upper airway. Thus, any effect that IMT may have had on the upper airway dilator muscles of these healthy participants was probably lost within the normal biological variation of upper airway calibre. Accordingly, these data suggest that the detection of IMT-induced

changes in the upper airway may require the examination of more functional outcomes related to upper airway collapse.

That no changes were found during wakefulness cannot be extrapolated necessarily to sleep. According to the "balance of pressures" concept, airway patency depends on the balance between collapsing intraluminal pressures generated by negative intrathoracic inspiratory pressures and the dilating/stabilising forces of the upper airway muscles (Brouillette & Thach, 1979). Neural drive to the upper airway muscles is reduced during sleep (Sauerland & Harper, 1976), and in healthy asleep participants exposed to negative intraluminal pressure the resulting imbalance of pressures is sufficient to elicit obstructive events (King *et al.*, 2000). This imbalance of airway pressure and dilating forces is even more marked in patients with OSAS, in part because such patients have an augmented neural drive to the upper airway muscles during wakefulness, which is lost during sleep (Mezzanotte *et al.*, 1996). However, the reduced neural drive to upper airway muscles during sleep may also increase the relative importance, in terms of maintaining airway patency, of the intrinsic properties of the airway wall. If IMT exerts its influence on airway function by improving the passive tone of the upper airway dilators and hence increasing pharyngeal wall stiffness, it is likely that any reduction in the collapsibility of the upper airway would be most noticeable during sleep. In this respect, patients with OSAS may benefit most from chronic IMT because their pharyngeal wall is more compliant compared with healthy participants, even in the absence of neural influences (Isono *et al.*, 1997).

In summary this study demonstrated that lateral narrowing of the upper airway occurred at relatively low levels of inspiratory resistive loading (10% MIP), but did not exhibit a dose response relationship with increasing loads. Anteroposterior narrowing at the level of the laryngopharynx occurred at higher levels of inspiratory resistive loading (≥ 30% MIP). The MRI technique used in the present study was not sufficiently sensitive to detect between-day changes in upper airway morphology. The relatively large biological variability of upper airway calibre makes MRI a blunt instrument for examining chronic luminal changes. Further studies are needed to determine the effect of chronic IMT on upper airway muscle structure and function in healthy and patient populations.

CHAPTER 5

ACUTE EFFECT OF INSPIRATORY PRESSURE-THRESHOLD LOADING UPON AIRWAY RESISTANCE IN PEOPLE WITH ASTHMA

[Note: this chapter has been published in full in Respiratory Physiology and Neurobiology]

5.1. INTRODUCTION

The ability of a deep inhalation (DI) to modify lower airway calibre has been known for a number of years. Nadel and Tierney (1961) were the first to demonstrate a marked decrease in airway resistance (bronchodilation) in healthy subjects after a DI during a period of induced bronchoconstriction. They suggested that increased stretching of airway smooth muscle, caused by greater transluminal airway pressures, induced a change in the airway lumen diameter. The response to DI in people with asthma differs from that of healthy people. Typically, in people with asthma, DI results in either a diminished or absent bronchodilation or even an increase in airway resistance (Kapsali *et al.*, 2000; Brown & Mitzner, 2001; Salome *et al.*, 2003). The bronchoconstriction seen after DI may be explained by changes in the excitation-contraction mechanism of the airway smooth muscle (Fredberg *et al.*, 1997). Slowly cycling cross bridges may become 'latched', stiffer and possess low hysteresis. Under such conditions the airway is less responsive to stretch and, if airway hysteresis is low relative to parenchymal hysteresis, further airway narrowing after DI is likely (Burns *et al.*, 1985).

Another factor that may contribute to the magnitude of airway re-narrowing after a DI is the extent to which the airway dilates in response to the pressure generated by DI. In support of this Jensen *et al.* (2001) demonstrated that not only did the airways of patients with asthma dilate less after DI, they also re-narrowed more. It has previously been proposed that the opening of narrowed airways in people with asthma may require negative pressures in excess of those generated by DI (Gunst

et al., 1988). Recently it has been demonstrated that positive pressure inflation is able to reduce airway obstruction in asthma patients who are unable to do so with an active DI (Slats *et al.*, 2008). The authors suggest that the positive pressures may have applied a greater stretching force on the airway than that which can be achieved under physiological conditions (Slats *et al.*, 2008).

An alternative method of increasing airway stretch may be to breathe against an inertial inspiratory load, which may lead to a temporal dislocation between intra-airway pressure and pleural pressure as the inertial load is overcome. Studies of pressure-threshold inspiratory muscle training have observed improvements in FEV_1 (Weiner *et al.*, 1992) and peak expiratory flow rate post-IMT (Lima *et al.*, 2008). In addition, medication usage has been shown to reduce, suggesting an improvement in the severity of disease *per se* (Weiner *et al.*, 2002a). These data are therefore consistent with the notion that pressure-threshold loading may impart a unique stretching stimulus to the airway. If this is the case, acute inspiratory loading may modify the bronchoconstrictor response to DI in people with asthma.

The aim of this study was to determine the acute effect of DI and various inspiratory loads upon respiratory system resistance (R_{rs}) in people with moderate asthma. It was hypothesised that inspiratory loading, combined with DI would elicit either no change, or an acute decrease in R_{rs}.

5.2. METHODS

5.2.1. Participants

After local research ethics committee approval and written informed consent 9 non-smoking adults (1 female) with a physician diagnosis of asthma and a history of respiratory complications, volunteered to participate in the study. Participants were being prescribed with medication to control their asthma symptoms. All participants were required to demonstrate an obstructive ventilatory defect as defined by a reduced FEV_1/FVC ratio below the 5^{th} percentile of the predicted value (Pellegrino *et al.*, 2005). Further, participants were also required to demonstrate either no response, or an increase in airway resistance, in response to a DI. Spirometry was undertaken after treatment withdrawal of 24 and 48 h for short- and long-acting medication, respectively. The severity of lung function impairment was classified as "moderate" based on the FEV_1 expressed as a percentage of the predicted value (Pellegrino *et al.*, 2005). Descriptive characteristics of the participants are shown in Table 5.1.

5.2.2. Procedures

Protocol

Measurements were made with the airway in its normal basal state of tone, i.e., not after induced bronchoconstriction. The DI and inspiratory loads were randomised and carried out on separate days. Tests were arranged to ensure that abstention from medication did not exceed 48 h, with a minimum of 48 h between consecutive tests. The same time of day was used for all tests to minimise the effect of circadian influences upon airway function (Mortola, 2004).

Prior to the baseline measurement of R_{rs}, lung volume history was monitored for 5 min by asking participants to breathe quietly on a mouthpiece connected to an electronic spirometer (Microloop, Micro Medical Ltd, Rochester, Kent, UK), whilst refraining from taking any DIs. Tidal volume loops were visually inspected in real time on the interfaced PC. Any evidence of DI during this period resulted in the 5 min period restarting. Only when participants had breathed for 5 min in the absence of DI were the baseline measurements made. Respiratory system resistance was measured immediately after the 5 min period and this was followed by either an unloaded DI, or a DI against one of the four loads. Respiratory resistance was measured immediately after each of the manoeuvres to determine any effect. The protocol was repeated three times using the same load, each separated by a 5 min period of quiet breathing with no DIs.

Lung and inspiratory muscle function

Lung function measurements were conducted in the sitting position using the electronic spirometer connected to a PC running Spida 5 software (Micro Medical Ltd). Measurements were performed and interpreted according to ATS/ERS guidelines (Miller *et al.*, 2005; Pellegrino *et al.*, 2005). Procedural details are described in section 2.2.2.

Inspiratory muscle function measurements were also conducted in the sitting position using a portable hand held mouth pressure meter (Micro RPM, Micro Medical Ltd, Rochester, Kent, UK). Maximum inspiratory mouth pressure (MIP) was determined from residual volume (RV). The maximum average pressure

sustained over one second from three manoeuvres that varied by less than 10% was recorded (ATS/ERS, 2002). Procedural details are described in section 2.2.3.

Table 5.1. Descriptive characteristics of the participants

Parameter	Mean ± SD	% Predicted
n (male/female)	8/1	-
Age (y)	38 ± 15	-
Stature (m)	1.75 ± 0.07	-
Body Mass (kg)	85.4 ± 18.8	-
FEV_1 (L)	3.18 ± 0.72	82 ± 12
FVC (L)	5.10 ± 1.13	109 ± 12
FEV_1/FVC (%)	63 ± 6	78 ± 7
FEF_{25-75} ($L \cdot s^{-1}$)	1.90 ± 0.60	43 ± 10
PEF ($L \cdot s^{-1}$)	8.48 ± 2.0	93 ± 16
MIP (cmH_2O)	123 ± 40	124 ± 33

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25-75}, forced expiratory flow between 25 and 75% of FVC; PEF, peak expiratory flow; MIP, maximum inspiratory mouth pressure. Predicted values for lung and respiratory muscle function were determined from the equations derived by (Quanjer *et al.*, 1993) and (Wilson *et al.*, 1984), respectively. Values are group means ± SD.

Airway resistance

Respiratory system resistance (R_{rs}) was measured using the forced oscillation technique (FOT; Quark i2m, Cosmed, Rome, Italy) in accordance with ERS recommendations (Oostveen *et al.*, 2003). A low amplitude pseudorandom multi-frequency pressure oscillation (4 – 48 Hz) was applied at the mouth during quiet tidal breathing. Participants wore a nose clip and supported their cheeks and floor of the mouth with their hands to minimise upper airway shunt due to compliance of these structures during the measurement. Equipment was calibrated before each session, using a calibration cylinder of known resistance, according to manufacturer's guidelines. Measurements at the 8 Hz frequency were recorded as

this frequency is most representative of changes in airway resistance (R_{aw}; Jensen *et al.*, 2001). Procedural details are described in section 2.2.7.

Inspiratory manoeuvres

Participants performed one of five different inspiratory manoeuvres at each visit. All manoeuvres were performed from RV and participants were instructed to inspire forcefully to fill their lungs as close to total lung capacity (TLC) as possible. Upon reaching TLC participants then relaxed to allow lung volume to return to functional residual capacity (FRC). Inspiratory manoeuvres were designed to assess the influence of a typical IMT protocol (50% MIP), as well as a low absolute inspiratory load (25 cmH_2O). One condition also controlled for the direct influence of hypocapnia upon the airway. Accordingly, the manoeuvres consisted of: 1) a single unloaded DI; 2) a single DI against a load of 25 cmH_2O; 3) a single DI against a load equivalent to 50% MIP (62 ± 20 cmH_2O); 4) 30 DIs at 50% MIP; 5) a repeat of trial 4 under conditions designed to maintain end-tidal carbon dioxide ($P_{ET}CO_2$) within the normocapnic range. Specifically, normocapnia was maintained by enclosing the inspiratory and expiratory ports of the threshold loading device within a 10 L re-breathing bag. A number of perforations were made in the bag to allow participants to re-breathe a mixture of atmospheric and expired air. A familiarisation session served to individualise the number of perforations required for each participant. During the 30 DI protocol, expired gas composition was analysed breath by breath using a metabolic measurement system (Oxycon Pro, Jaeger, Hoechberg, Germany). The effectiveness of this method was determined by paired-samples *t*-test, which

revealed no significant difference between resting and loaded breathing $P_{ET}CO_2$ (36.09 ± 1.59 vs. 36.30 ± 2.84 mmHg; P = 0.70). Loaded breathing was undertaken using a calibrated pressure-threshold device (POWERbreathe®, HaB Ltd, Southam, UK; Caine & McConnell, 2000).

5.2.3. Statistical Analyses

Stability of baseline R_{rs} (8 Hz) over all trials was assessed with one-way repeated-measures analysis of variance (ANOVA). Test-retest correlations were derived from the ANOVA as intraclass correlation coefficients (ICCs).

Repeated measures ANOVA was used to examine group mean differences in R_{rs} (8 Hz) between "inspiratory load" (single unloaded DI; single DI at 25 cmH_2O; single DI at 50% MIP; 30 DIs at 50% MIP; 30 DIs at 50% MIP [normocapnic]) and "response to load" (pre-DI, post-DI). Mauchly's sphericity test was used to check homogeneity of covariance and violations of this assumption were corrected using the Greenhouse-Geisser adjustment. Following significant main effects, planned pairwise comparisons were made using the Bonferroni method. Statistical significance was defined as $P < 0.05$.

5.3. RESULTS

5.3.1. Baseline Respiratory System Resistance and Response to Acute Inspiratory Loading

Repeated-measures ANOVA revealed no significant between-trial differences in baseline R_{rs}. Test-retest reliability revealed an intraclass correlation coefficient of 0.87 (95% likely range: 0.71-0.96). R_{rs} increased by 15.7 ± 11.0% in response to DI (3.7 ± 1.8 vs. 4.2 ± 1.7 hPa·L·s^{-1}; *P* = 0.016) and 20.8 ± 26.1% in response to a single inhalation at 25 cmH_2O (3.5 ± 1.7 vs. 4.0 ± 1.5 hPa·L·s^{-1}; *P* = 0.03). No increase was observed in response to a single inhalation at 50% MIP (3.6 ± 1.6 vs. 3.6 ± 1.5 hPa·L·s^{-1}; *P* = 0.95), to 30 inhalations at 50% MIP (3.9 ± 1.5 vs. 4.2 ± 2.0 hPa·L·s^{-1}; *P* = 0.16) or to 30 inhalations at 50% MIP under normocapnic conditions (3.9 ± 1.5 vs. 3.9 ± 1.5 hPa·L·s^{-1}; *P* = 0.55; Figure 5.1 and 5.2).

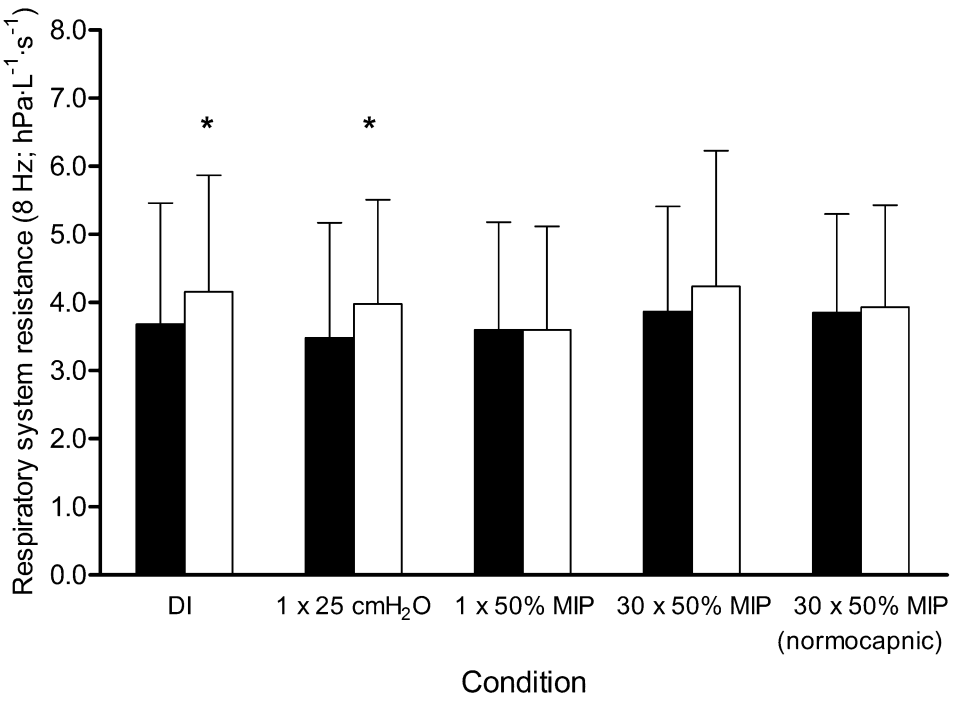

Figure 5.1. Respiratory system resistance (R_{rs}) in response to various inspiratory manoeuvres. Closed bars = baseline R_{rs}; open bars = post-manoeuvre R_{rs}. ***** *P* < 0.05, significantly from baseline. Values are group means ± S.D.

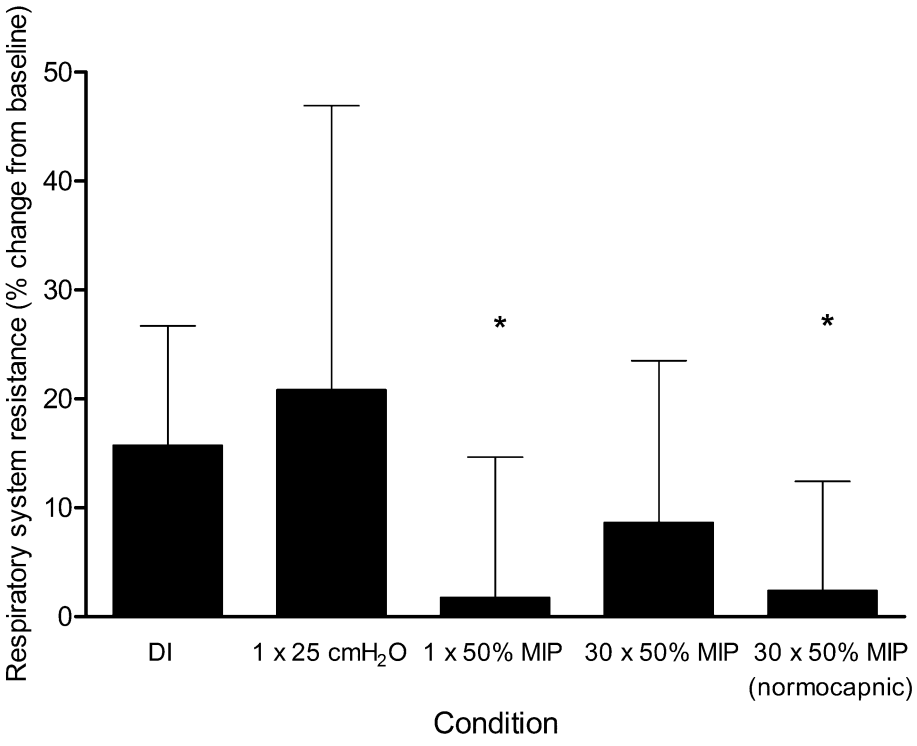

Figure 5.2. Percent change from baseline in respiratory system resistance (R_{rs}) in response to various inspiratory manoeuvres. ***** $P < 0.05$, significantly different from DI. Values are group means ± S.D.

5.4. DISCUSSION

5.4.1. Main Findings

The finding of increased R_{rs} after DI agrees with previous studies in people with asthma (Brown *et al.*, 2001; Salome *et al.*, 2003). The attenuation of this response, seen after both a single loaded breath at 50% MIP and 30 loaded breaths at 50% MIP, is new. Furthermore, the magnitude of the latter response appears to be modulated by changes in airway carbon dioxide (CO_2) during loaded breathing. The attenuated response to DI was not seen during a single breath at 25 cmH_2O. These data confirm the hypothesis, but suggest that the pressure-threshold load required to modify the response to DI in people with asthma must exceed 25 cmH_2O. Further, they suggest that there is a confounding influence of airway CO_2 upon the response to loaded breathing.

Baseline R_{rs} did not differ between trials, which supports the effectiveness of the volume history control prior to each of the measurements. It was decided to monitor tidal breathing for 5 minutes, which is shorter than some previous studies (20 min; Scichilone *et al.*, 2001), but longer than others (1 min; Burns & Gibson, 2001). Those studies that have monitored volume history for longer than 5 minutes have done so to minimise the impact on their measurement of a preceding forced ventilatory manoeuvre. Although the measurements were not preceded by forced manoeuvres caution was taken and monitoring of volume history was extended beyond that normally recommended, i.e., 3 minutes (Oostveen *et al.*, 2003).

The response of R_{rs} to DI in people with asthma differs from that of healthy people (constriction vs. dilation, respectively). As has been observed previously, participants in this study also showed an increase in R_{rs} in response to DI (Kapsali *et al.*, 2000; Brown *et al.*, 2001; Salome *et al.*, 2003). The mechanisms underpinning this abnormal response of airway smooth muscle to DI are unknown, but it has been suggested that the airway smooth muscle (ASM) of people with asthma adopts a so-called 'latched' state (Fredberg *et al.*, 1999), in which it is stiffer and less compliant, resulting in a less distensible airway. This renders the airway less responsive to the stretching influence of the surrounding parenchyma during lung inflation. Under these conditions the forces generated by normal lung inflation are thought to be insufficient to elicit ASM relaxation in response to DI, with the ability to dilate the airway related to the severity of the asthma symptoms (Jensen *et al.*, 2001). In addition, it has also been shown that the extent of airway re-narrowing after DI is inversely correlated with the extent to which the airways are able to dilate (Salome *et al.*, 2003).

Evidence to support this notion was provided by a series of experiments in which intra-breath airway resistance was measured using a modified forced oscillation technique. Jensen and colleagues (2001) noted that the minimum R_{aw} achieved by people with mild/moderate and severe asthma at TLC during a DI was significantly lower in people with asthma, compared to healthy people. There was also an inverse dose response relationship between the R_{aw} at TLC and the severity of asthma. Furthermore, in the presence of a bronchoconstrictor, this difference between groups (healthy, mild/moderate asthma, severe asthma)

increased, and after a bronchodilator, the difference diminished. Thus, in both health and disease, the ability to stretch the airways during a DI appears to be dependent upon the baseline level of airway stiffness due to the contractile state of the airway smooth muscle.

In this respect it has recently been demonstrated that positive pressure inflation reduces airway obstruction in asthma patients who are unable to do so with an active DI (Slats *et al.*, 2008). The authors suggest that the positive pressures may have applied a greater stretching force on the airway than that which can be achieved under physiological conditions (Slats *et al.*, 2008). The positive pressure model is also suggested to prevent airway wall oedema, which may occur during negative pressure inflation. Burns and Gibson (2002) suggest that the increase in resistance seen in their subjects may have been a result of fluid leaking into the airway wall with a consequent reduction in airway calibre. An alternative explanation however, is that the increase in resistance was due to acute airway hypocapnia after the repeated DIs, something controlled for in this study.

This functional approach to examining the influence of DI upon the ability to stretch the airways supports the notion that a normal DI provides an inadequate stimulus to the stiffened airway smooth muscle of people with asthma. The data presented in this study are consistent with this notion; both DI and a loaded breath at 25 cmH_2O were associated with bronchoconstriction, whilst a moderate (50% MIP) pressure-threshold load abolished the bronchoconstrictor response to DI.

Whilst this study is not the first to examine the effect of added resistances upon the response to DI, it is the first to do so in people with asthma. Two previous studies on healthy people have examined the influence of elastic inspiratory loads in the form of chest wall strapping (CWS; Duggan *et al.*, 1990; Torchio *et al.*, 2006). In the first of these, Duggan *et al*. (1990) demonstrated that the bronchodilatory effect of DI was dependent upon either, achieving an inspiratory tidal volume (V_T) > 68% of TLC, or by combining a smaller V_T (56% of TLC) with the increased transpulmonary pressure generated by CWS. The authors also noted a slightly greater response to fast DI than to slow DI (though not significant), which would be consistent with the production of slightly elevated transluminal airway pressure at raised inspiratory flow rates. The authors concluded that the primary determining factor for the magnitude of the response to DI was the resultant transluminal airway pressure (Duggan *et al.*, 1990). In contrast, a more recent study by Torchio *et al*. (2006) observed a concomitant reduction in the bronchodilator effect of DI under conditions of CWS, which occurred in the presence of equivalent transpulmonary pressure (albeit at a lower end inspiratory lung volume (EILV)). The discrepancy between the findings of these two studies is most likely explained by the differing severity of CWS employed by each. For the participants in the Torchio study, the strapping resulted in a decrease in TLC of only around 12%, whereas the strapping imposed in the Duggan study reduced TLC by 43%. Accordingly, the transpulmonary pressures achieved in the Duggan study were almost twice those of the Torchio study (~11cmH_2O vs. ~19 cmH_2O).

The importance of attaining a critical transpulmonary pressure in order to elicit a bronchodilatory response to DI is supported by evidence from a canine model (Brown & Mitzner, 2001). Varying magnitudes of lung inflation were imposed upon anaesthetised, paralysed dogs receiving methacholine by continuous infusion. The authors noted that lung inflations generating airway pressures less than 35 cmH_2O induced bronchoconstriction, whilst inflation generating airway pressures of 45 cmH_2O induced bronchodilation. However, since EILV was proportional to inflation pressures, it is difficult to separate the influence of airway stretch due to differences in lung volume, and the effects of differing transpulmonary pressure in this model.

In the absence of an externally imposed load, transpulmonary pressure is a function of inspired volume. Salerno and colleagues (2005) examined the influence of differing inspired volumes upon the response to DI in healthy participants under the influence of methacholine-induced bronchoconstriction. They noted that, despite a non-linear relationship between transpulmonary pressure and EILV, the response to DI was related linearly to EILV. They argued that this provided evidence that the primary stimulus to the influence of DI upon ASM was stretching, and not transpulmonary pressure. To the best of our knowledge, this study is the first to impose a resistance at the mouth, but in common with Brown and Mitzner's (2001) dog model, also found that the bronchoconstrictor response to DI was only modified by a load in excess of 25 cmH_2O. Since higher threshold loads are associated with lower EILV (because of the length-tension relationship of the inspiratory muscles), these data are

consistent with those of Duggan and colleagues (1990), suggesting that transluminal pressure and airway stretch (resulting from volume-related parenchymal pull on the airways) have discrete influences, acting in concert to generate ASM relaxation.

The inverse relationship of lung volume and airway resistance has long been known, with higher airway resistance seen at lower lung volumes (Briscoe & Dubois, 1958). Indeed, a reduction in FRC after the unloaded DI may explain the increase seen in airway resistance for this experimental condition. However, a change in operating lung volume of approximately 2 litres would be required to explain the absence of this response after a 50% MIP load (Brown *et al.*, 2007). It therefore seems very unlikely that changes in FRC explain any of the observations reported in this study.

Overall, these data suggest that in the presence of spontaneous airway tone, people with moderate asthma are unable to elicit a bronchodilation in response to DI; indeed, they show a bronchoconstrictor response. However, when transpulmonary pressure is increased by imposition of an adequate inspiratory load (50% MIP), and combined with a DI, the response of the ASM is shifted towards normality, i.e., there is neither bronchodilation nor constriction.

The nature of the pressure-threshold load utilised in the present study is worthy of brief comment, since its inertial properties may be central to the nature of the distorting influence imposed upon the ASM. The pressure-threshold valve does

not open until intra-thoracic pressure equals the valve opening pressure. This means that there is a brief period at the onset of the inspiratory effort, before the valve opens, when there is intrathoracic gas decompression, which presumably applies a brief stretching influence upon the airways. Once the valve opens, air enters the airways and lung inflation occurs, The dynamics of these changes in relation to the mechanical forces exerted upon the ASM are unknown, but worthy of further exploration, as they may provide a potent method of distorting ASM and inducing relaxation.

One of the original rationales for this study was to assess whether improvements in FEV_1 in people with asthma after inspiratory muscle training (Weiner *et al.*, 1992) might be due to the chronic effect of inspiratory pressure-threshold loading upon the latched state of the ASM. Accordingly, the response to single loaded breaths, as well as multiple breaths that simulated a typical inspiratory muscle training protocol was assessed. It was found that the response to 30 breaths at the 50% MIP load was modulated by the prevailing airway CO_2, such that hypocapnia attenuated the effect of the inspiratory load. The bronconstrictive effect of hypocapnia is well known (see Bruton & Holgate, 2005), and when hypocapnia was prevented during the 30 breath condition, the attenuation of the bronchoconstrictor effect of DI was enhanced. These data therefore suggest that hypocapnia may exert an independent effect upon ASM, which could reduce the beneficial effect of raised transluminal airway pressure generated by pressure-threshold loading at 50% MIP. This observation may be important in terms of the hypothesised effect of inspiratory muscle training upon the latched state of ASM,

i.e., the influence of the raised transluminal airway pressure induced by the inspiratory loading upon ASM may be dependent upon maintenance of normocapnia. It is also noteworthy that the acute effect of 30 loaded breaths was no greater than the effect of 1 breath.

In summary, this study has shown that the spontaneous bronchoconstrictor tone of people with moderate asthma is increased by DI, and by an externally imposed inspiratory load of 25 cmH_2O. This bronchoconstrictor response was abolished when the load was increased to 50% of MIP. Finally, during multiple loaded breaths at 50% of MIP that simulated an inspiratory muscle training session, the attenuation of the bronchoconstrictor response was enhanced when airway CO_2 was controlled by re-breathing.

CHAPTER SIX

EFFECT OF SIX WEEKS INSPIRATORY PRESSURE-THRESHOLD LOADING UPON AIRWAY FUNCTION AND SENSATION OF BREATHING EFFORT IN PEOPLE WITH ASTHMA

6.1. INTRODUCTION

Previous studies have demonstrated improvements in asthma symptoms, medication usage, forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV_1) after a period of inspiratory muscle training (IMT; Weiner *et al.*, 1992). The improvements in asthma symptoms after IMT have been attributed to the increased strength of the inspiratory muscles and a concomitant reduction in respiratory muscle effort sensation and dyspnoea (McConnell & Romer, 2004). In this respect, Weiner and colleagues (2002a) have shown a close correlation between the IMT-induced improvement in inspiratory muscle strength and the reduction in breathing effort sensation during a loaded breathing task. In addition, they have demonstrated that improvements in inspiratory muscle strength are correlated with the magnitude of the reduction in medication consumption following IMT in patients with mild/moderate asthma (Weiner *et al.*, 2002a). However, the mechanism(s) by which improvements in FVC and FEV_1 arise after IMT is unknown, but may be associated with an improved ability to achieve a higher starting lung volume after IMT. Under these conditions, the increased parenchymal pull on the airways would permit higher flow rates to be achieved, and thus, an improvement in FVC, FEV_1 and peak expiratory flow. An alternative explanation is that the deep inhalations and pressure-threshold loading associated with IMT may induce substantial stretching of the airway smooth muscle (ASM), due to the increase in transluminal airway pressure, prompting the smooth muscle to adopt a more relaxed resting state.

In a previous chapter (Chapter 5) the acute response to a deep inhalation (DI) was examined in people with asthma, as well as the influence of pressure-threshold loading and an acute bout of IMT. It was hypothesised that IMT induces a relaxation of the ASM via increased transluminal airway pressure concomitant to a volume-related parenchymal pull on the ASM. Using the forced oscillation technique (FOT) to measure respiratory system resistance (R_{rs}) it was demonstrated that single and multiple breaths against a pressure-threshold load equivalent to 50% of maximal inspiratory pressure (MIP) abolished the increase in R_{rs} typically seen after an unloaded DI. This finding adds support to the hypothesis that IMT may exert its effect by invoking an acute stretching of the ASM beyond that achievable under physiologic conditions. If this is the case it is reasonable to postulate that a period of chronic IMT may be able to 'un-latch' the stiffer ASM of people with asthma and normalise airway function and the response to DI. This mechanism may also provide an explanation for improvements in airway function following IMT seen in previous studies (Weiner *et al.*, 1992; McConnell *et al.*, 1998).

The aim of this study was to determine the effect of six weeks IMT on R_{rs} and other indices of airway function in people with mild asthma. In addition, the response to DI and perception of breathing effort were also investigated.

6.2. METHODS

6.2.1. Participants

After local ethics committee approval and written informed consent 8 non-smoking adults (4 F and 4 M) with a physician diagnosis of asthma and a history of respiratory complications, volunteered to participate in this study. Participants had stable asthma and were free of any recent chest infection or allergy at the time of testing, which took place outside of the pollen season. All participants used β_2-agonist inhaler medication, on an as needed basis, to control their asthma symptoms. Two participants also used inhaled corticosteroids. All of the participants were required to demonstrate an obstructive ventilatory defect as defined by a reduced FEV_1/FVC ratio below the 5^{th} percentile of the predicted value (Pellegrino *et al.*, 2005). Further, participants were also required to demonstrate either no response, or an increase in airway resistance, in response to a DI. All measurements were undertaken after treatment withdrawal of 24 and 48 h for short- and long-acting medications, respectively. Users of inhaled corticosteroids withdrew treatment 72 h prior to testing. The severity of lung function impairment was classified as "mild" based on the FEV_1 expressed as a percentage of the predicted value (Pellegrino *et al.*, 2005). Descriptive characteristics of the participants are shown in Table 6.1.

Table 6.1. Descriptive characteristics of the participants

Parameter	Mean Value	% Predicted
n (male/female)	4/4	-
Age (y)	38 ± 14	-
Stature (m)	1.68 ± 0.11	-
Body Mass (kg)	73.9 ± 17.7	-
FEV_1 (L)	2.50 ± 0.79	76 ± 17
FVC (L)	3.95 ± 1.09	100 ± 15
FEV_1/FVC (%)	63 ± 6	78 ± 7
FEF_{25-75} ($L \cdot s^{-1}$)	1.90 ± 0.60	43 ± 10
PEF ($L \cdot s^{-1}$)	6.41 ± 2.41	81 ± 21
MIP (cmH_2O)	98 ± 21	113 ± 11
R_{rs} ($hPa \cdot L \cdot s^{-1}$)	4.51 ± 1.83	151 ± 54

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25-75}, forced expiratory flow between 25 and 75% of FVC; PEF, peak expiratory flow; MIP, maximum inspiratory mouth pressure; R_{rs}, respiratory system resistance. Predicted values for lung, respiratory muscle function and respiratory resistance were determined from the equations derived by Quanjer *et al*., (1993), Wilson *et al*., (1984), and Pasker (1996). Values are mean ± SD.

6.2.2. Procedures

Study design

The study incorporated a repeated measures, placebo controlled cross-over design. Participants visited the laboratory at baseline, after a 3 week placebo intervention and again after 6 weeks inspiratory muscle training. Testing was ordered so that a prior test would not impact on the result of a subsequent test. Specifically, baseline R_{rs} and response to DI (Figure 6.1.) was conducted first, followed by measurement of F_ENO, lung function, respiratory muscle function and assessment of breathing effort.

Figure 6.1. Protocol to assess the effect of a deep inhalation on airway resistance. The protocol was repeated after 3 wk placebo and 6 wk IMT. DI: deep inhalation, FOT: forced oscillation technique.

Respiratory system resistance and response to deep inhalation

Respiratory system resistance (R_{rs}) was measured using the forced oscillation technique (FOT; Quark i2m, Cosmed, Rome, Italy) in accordance with European Respiratory Society recommendations (Oostveen *et al.*, 2003). A low amplitude pseudorandom multi-frequency pressure oscillation (4-48 Hz) was applied at the mouth during quite tidal breathing. Measurements at the 8 Hz frequency were recorded as this frequency is most representative of changes in airway resistance (R_{aw}; Jensen *et al.*, 2001). Three measurements were taken immediately before and after a DI to TLC. The mean value of the three measurements was calculated and recorded. Procedural details are described in section 2.2.7.

Exhaled Nitric Oxide

The inflammatory status of the airway, an indicator of asthma severity, was determined by the measurement of the fractional concentration of nitric oxide in expired air (F_ENO). Exhaled NO was measured by a chemiluminescence analyser (Sievers NOA-280i, Sievers Instruments, Boulder, CO) according to current recommendations (ATS/ERS, 2005). Procedural details are described in section 2.2.6.

Lung function

Lung function measurements were conducted in the sitting position using an electronic spirometer (Microloop, Micro Medical Ltd, Rochester, Kent, UK) connected to a PC running Spida 5 software (Micro Medical Ltd, Rochester, Kent, UK). Measurements were performed and interpreted according to ATS/ERS

guidelines (Miller *et al.*, 2005; Pellegrino *et al.*, 2005). Procedural details are described in section 2.2.2.

Respiratory muscle function

Maximum inspiratory mouth pressure (MIP) and maximum expiratory mouth pressure (MEP) were determined from residual volume (RV) and total lung capacity (TLC), respectively, using a portable hand held mouth pressure meter (Micro RPM, Micro Medical Ltd, Rochester, Kent, UK). The maximum average pressure sustained over one second from three manoeuvres that varied by less than 10% was recorded (ATS/ERS, 2002). Procedural details are described in section 2.2.3.

Incremental threshold loading test

Breathing effort was assessed whilst participants breathed through a bespoke calibrated pressure-threshold device against 4 progressive loads (inspiratory mouth pressures of 10, 20, 30 and 40 cmH_2O). Breathing pattern was self-paced and after breathing for 1 min at each load participants rated their sensation of breathing effort using the Borg CR-10 scale (Borg, 1982). The appropriateness of this scale to measure respiratory sensations in people with asthma has previously been demonstrated (Burdon *et al.*, 1982). Procedural details are described in section 2.2.8.

Inspiratory muscle training

Participants performed 30 dynamic inspiratory efforts, using a pressure-threshold inspiratory muscle training device (POWERbreathe®, HaB Ltd, Southam, UK), twice daily for 6 weeks. Participants were instructed to inspire fully and with maximal effort from RV to TLC. At the start of the training the inspiratory load was set to 50% of MIP. This load has previously been shown to result in improvements in MIP (Romer *et al.*, 2002b; Romer & McConnell, 2003). Participants were instructed to periodically increase the load to a level that would only just allow them to complete the 30 manoeuvres. Participants completed training diaries for the duration of the study to monitor compliance with the training programme. Procedural details are described in section 2.2.4.

Placebo intervention

To generate an expectation similar to that after IMT participants also undertook a placebo intervention prior to the IMT phase of the study. Participants were told that the effects of the medications were being compared with those of the IMT. The placebo consisted of a non-pharmacological metered dose inhaler (HFA Placebo Aerosol, Vitalograph, Buckingham, UK) containing only propellant. Participants were instructed to perform 2 actuations per day (am and pm) for 3 weeks in addition to their usual medication. On completion of the study participants were informed of the deception in writing (See appendix A-6).

6.2.3. Statistical Analyses

Stability of baseline R_{rs} was assessed by calculating the within-subject coefficient of variation (CV). Repeated measures ANOVA was used to examine group mean differences across all airway and respiratory muscle function variables, across all conditions. Mauchly's sphericity test was used to check homogeneity of covariance and violations of this assumption were corrected using the Greenhouse-Geisser adjustment. Following significant main effects, planned pairwise comparisons were made using the Bonferroni method. Statistical significance was defined as $P < 0.05$.

6.3. RESULTS

Dynamic lung function, inspiratory muscle function, and inflammatory status

No changes were detected in any of the lung function parameters after either the placebo or IMT phase of the intervention (FVC, $P = 0.08$; FEV_1, $P = 0.29$ and PEF, $P = 0.43$, Table 6.2 and Figure 6.2.). The inflammatory status of the airway, assessed by F_ENO, did not differ between visits ($P = 0.09$).

MIP increased by 28% compared to baseline after 6 wk IMT (98 ± 21 vs. 125 ± 28 cmH_2O, $P < 0.001$). No changes where detected in MIP or MEP after placebo (Table 6.2.).

Table 6.2. Lung and respiratory muscle function at baseline, post placebo and pre- and post-IMT. Data are group mean ± SD

Parameter	Baseline	Post placebo	Post IMT
$R_{rs}(hPa \cdot L \cdot s^{-1})$	4.51 ± 1.83	4.69 ± 1.65	4.62 ± 1.54
Response to DI (ΔR_{rs} (%))	24.1 ± 28.7	16.1 ± 11.6	9.8 ± 6.2
FEV_1 (L)	2.50 ± 0.79	2.39 ± 0.89	2.50 ± 0.75
FVC (L)	3.95 ± 1.09	3.83 ± 1.21	4.00 ± 1.08
PEF $(L \cdot s^{-1})$	6.41 ± 2.41	6.15 ± 2.41	6.6 ± 1.94
NO (ppb)	41.7 ± 28.4	48.9 ± 36.2	46.6 ± 29.7
MIP (cmH_2O)	98 ± 21	97 ± 21	125 ± 28*
MEP (cmH_2O)	116 ± 22	122 ± 21	118 ± 20

NO, Nitric Oxide; MEP, maximum expiratory mouth pressure, *Significantly different from baseline, $P < 0.001$.

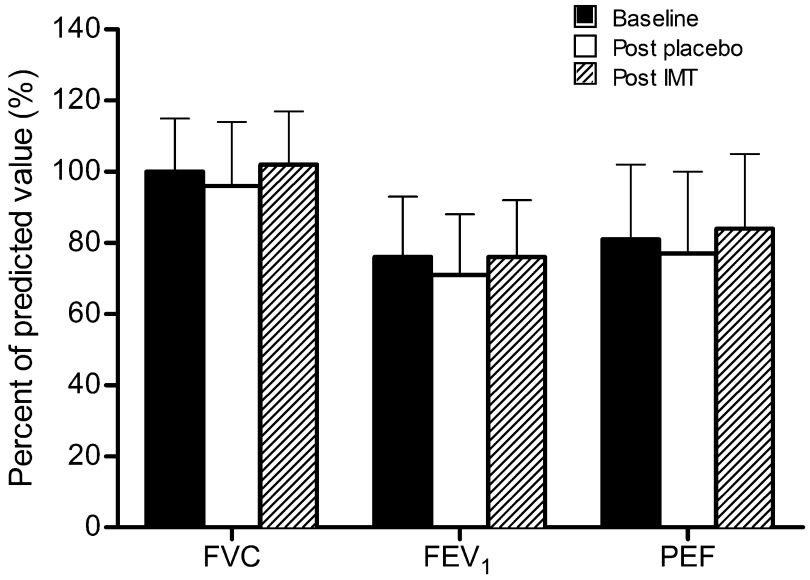

Figure 6.2. Lung function indices at baseline, post placebo, and post IMT. Predicted values were determined from the equations derived by Quanjer *et al*. (1993). Values are mean ± SD.

Baseline airway resistance and response to deep inhalation

Test-retest reliability between pre and post-placebo revealed a technical error of measurement of 0.53 $hPa \cdot L \cdot s^{-1}$ and a coefficient of variation (CV) of 11.4%. With 8 participants this study had sufficient power (80%) to detect a 17% change in R_{rs}. To detect a 5% change in R_{rs} with power of 80% would require 90 participants. Repeated-measures ANOVA revealed no differences in the response to DI (P = 0.76) across the 3 trials (Table 6.3; Figure 6.3).

Table 6.3. Respiratory system resistance (R_{rs}) response to DI at baseline and pre- and post- placebo and IMT, respectively.

Subject	R_{rs} ($hPa \cdot L \cdot s^{-1}$)								
	Baseline			Post-Placebo			Post IMT		
	Pre-DI	Post-DI	% Change	Pre-DI	Post-DI	% Change	Pre-DI	Post-DI	% Change
1	3.19	3.51	9.9	3.11	3.24	4.1	3.28	3.53	7.6
2	4.76	4.88	2.4	5.72	7.27	27.0	5.24	6.07	15.8
3	5.88	7.81	32.8	6.67	7.86	17.9	6.19	6.62	6.8
4	3.49	3.67	5.1	3.58	3.62	1.2	3.57	3.79	6.1
5	1.99	3.63	82.3	2.09	2.66	27.6	2.27	2.39	5.3
6	7.88	7.97	1.1	6.51	8.13	24.7	6.60	8.07	22.1
7	5.14	7.60	47.8	5.14	6.38	24.0	5.71	5.96	4.4
8	3.73	4.14	11.0	4.72	4.83	2.3	4.11	4.54	10.4
Mean	4.51	5.40	24.1	4.69	5.50	16.1	4.62	5.12	9.8
SD	1.83	2.03	28.7	1.65	2.19	11.6	1.54	1.88	6.2

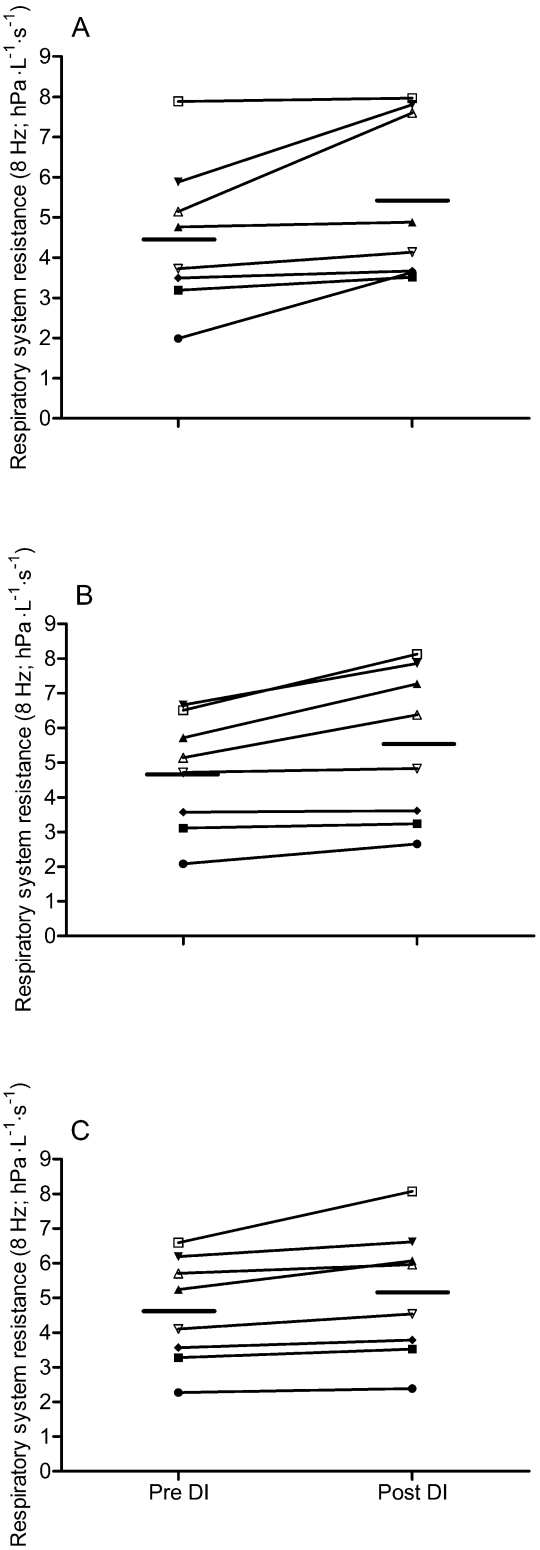

Figure 6.3. Individual and group mean (bars) responses to deep inhalation (DI) at baseline (A), post placebo (B) and post IMT (C).

Perception of breathing effort

Perception of breathing effort (RPE) was reduced across all breathing loads after IMT (10 cmH_2O 2.3 ± 0.7 vs. 0.5 ± 0.5, $P < 0.001$; 20 cmH_2O, 3.7 ± 0.6 vs. 1.9 ± 0.8, $P < 0.004$; 30 cmH_2O, 4.9 ± 1.5 vs. 2.9 ± 0.7, $P < 0.05$; 40 cmH_2O, 6.3 ± 1.8 vs. 3.7 ± 0.8; $P < 0.01$; Figure 6.4). No changes were seen after placebo except at the 10 cmH_2O load (2.3 ± 0.7 vs. 1.5 ± 1.0, $P < 0.005$).

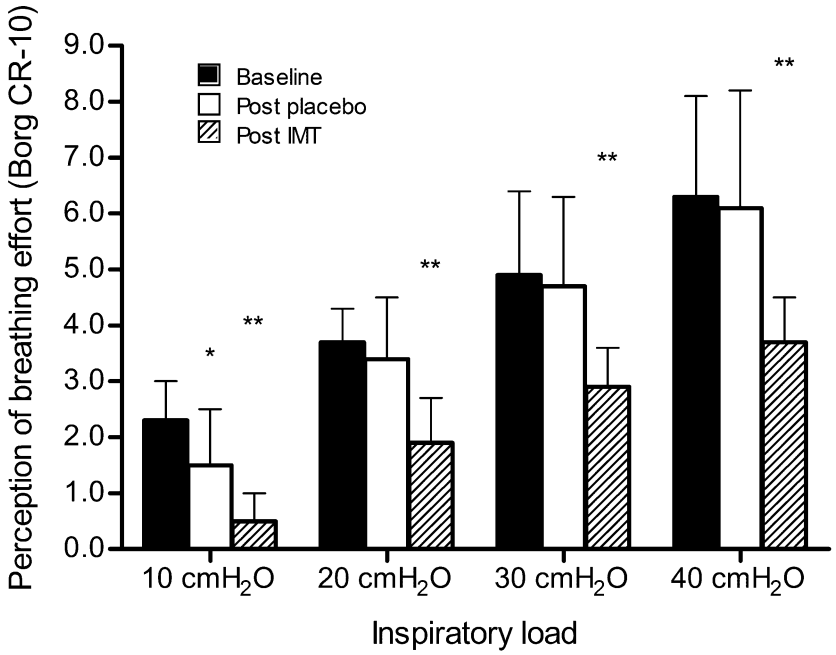

Figure. 6.4. Perception of breathing effort at baseline, post placebo and post IMT. Data are group mean ± SD. * significantly different from baseline, $P < 0.05$; ** significantly different from baseline and placebo, $P < 0.05$.

The relationship between changes in MIP and perception of breathing effort for all breathing loads are shown in Figure 6.5. There was a significant correlation between the relative change in MIP and respiratory effort sensation at the 10% MIP load ($r = -0.79$, $P < 0.05$) and a strong, but not significant correlation at 20

and 30 % MIP loads (r = -0.69, P = 0.06; r = -0.71, P = 0.05). The strength of the relationship at 40%, was much weaker (r = -0.34, P = 0.42).

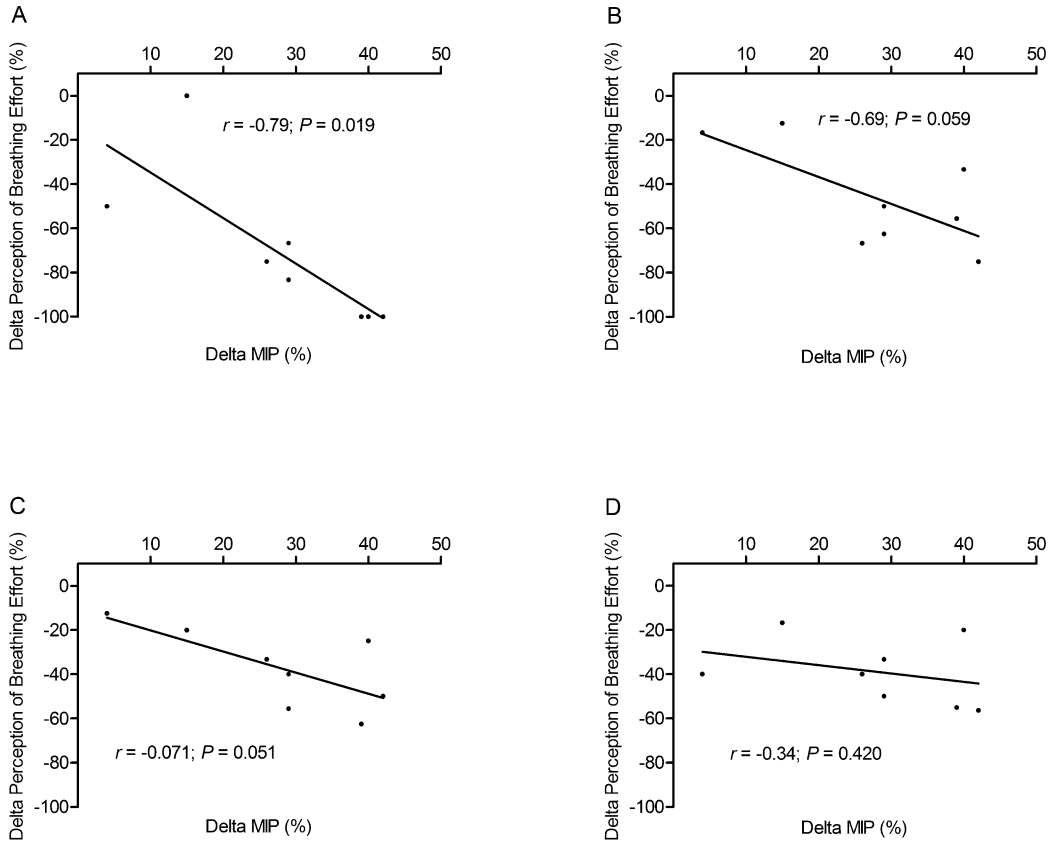

Figure 6.5. Change in maximum inspiratory pressure (MIP) versus change in perception of breathing effort (Borg CR-10) to an incremental threshold loading test at 10, 20, 30 and 40 cmH_2O (panels A, B, C, and D, respectively).

6.4. DISCUSSION

6.4.1. Main Findings

The aim of this study was to determine the effect of six weeks IMT upon indices of airway function, response to DI, and perception of breathing effort in people with mild asthma.

No changes were detected in either airway function or the response to DI after a 6 week period of IMT. However, perception of breathing effort was lowered across all breathing loads post IMT compared to baseline and placebo.

Airway function and response to deep inhalation

In Chapter 5, data was presented showing that single and multiple breaths at 50% MIP attenuated the increase in R_{rs} typically seen in people with asthma after a DI. This finding supports the notion that pressure-threshold loading imparts a stretching stimulus to the airway that is not achieved under physiological conditions. In this respect, it is likely that the load imposed during the 6 wk period of IMT (50% MIP), in the present study, provided sufficient stimulus to the airway. All reasonable precautions were taken to control for errors in the measurement of R_{rs}. Specifically, all measurements were arranged to ensure that prior tests did not influence a subsequent test. The measurement of baseline R_{rs} was only made after volume history had been monitored for 5 min to ensure the absence of any DI that may affect the measurement. The effectiveness of this approach was demonstrated in Chapter 5. Also, exhaled Nitric Oxide (F_ENO), measured to provide an indication of the inflammatory status of the airway,

remained stable for the duration of the study. It has previously been shown that the extent of airway inflammation can influence the response to DI (Pliss *et al.*, 1989). Finally, circadian influences were minimised by testing participants at the same time of day (Mortola, 2004).

However, analysis of baseline R_{rs} suggests that this study had sufficient power to detect a 17% change in baseline airway resistance 80% of the time (0.77 $hPa \cdot L \cdot s^{-1}$). Slats *et al.* (2008) recently demonstrated an acute 0.12 $hPa \cdot L \cdot s^{-1}$ (3%) reduction in baseline airway tone (i.e. not after induced bronchoconstriction) in response to an unloaded DI in people with moderate asthma who had an intact response to DI . A chronic change in R_{rs} is likely to be of smaller magnitude than an acute change; however, post-hoc analysis revealed that to detect a similar change to Slats *et al.* (2008) after 6 wk IMT, would have required 250 participants, which is clearly impractical. Thus, these data suggest that the spontaneous tone of the airway may not provide the magnitude of effect that is needed to detect between day changes in R_{rs}. However, the study by Slats *et al.*, (2008) reported a far greater response to DI (22%) when bronchoconstriction had been induced by methacholine inhalation, which may offer an alternative method of examining this response.

The strict control employed in the measurement of R_{rs} suggests that any effect IMT may have had upon the airway was small and lost in the inherent variability of airway tone. Accordingly, these data suggest that the detection of any IMT-induced changes in the airway may require alternative approaches that may include using an

animal model to examine isolated bronchial segments after a period of loaded breathing.

Aside from the sensitivity of the measure, the inability to detect a reduction in baseline airway function after IMT may have been a consequence of the duration of the training program. A six week intervention may not have been sufficient to induce a chronic effect that is reflected in improvements in airway function. Weiner *et al.* (1992) used a 6 month IMT intervention and found significant improvements in FEV_1 and FVC in their participants. Although the intervention used in the present study was only 6 wk (a typical IMT protocol in healthy people to improve inspiratory muscle function), there were similar improvements in MIP between this and the Weiner study (~ 28%). Further, a pilot study conducted by McConnell *et al.* (1998) revealed improvements in PEF and a trend toward improvements in FVC and FEV_1 after just 3 wk IMT, in participants with mild/moderate asthma. Despite this, it is possible that the time course of change in MIP differs from that of the ASM, and that to evoke a change in ASM, a longer intervention than that used in this study may be required.

Another explanation for the apparent improvement in airway function seen in the Weiner study may be that the improvements seen in FEV_1 were an artefact of an increased FVC that enabled a higher starting lung volume to be achieved post- compared to pre-IMT. A higher starting lung volume would increase the amount of stretch applied to the ASM by the parenchyma allowing higher flow rates to be generated. The present study found no differences in any of the forced ventilatory

volumes or flows between any of the conditions (Figure 6.2.). A reason for the differences between this study and Weiner *et al.* (1992) may have been due to differences in the breathing pattern employed during IMT between the two studies. The participants in the present study performed dynamic efforts from RV with a load equal to 50% MIP from the outset. Weiner *et al.* (1992) adopted a less demanding approach, and do not emphasise a forceful, rapid inhalation against the inspiratory load, as was done in the present study; this may minimise airway hypocapnia by slowing breathing frequency (see also below). In addition, for the first month of training their participants trained with a resistance of 15% MIP for 1 week followed by regular increments of 5% to end the first month at 60% MIP. Training then continued at 60% MIP for the remaining 5 months. It has previously been demonstrated that unloaded inspiratory manoeuvres performed at TLC can result in increases in FVC (Fanta *et al.*, 1983). The low inspiratory pressures used by Weiner *et al.* (1992) during the first 4 weeks of their study would have allowed their participants to either achieve, or come very close to achieving, TLC. Consequently the degree of inspiratory muscle shortening, and strengthening, at high lung volumes, would have been greater than that of the participants used in the present study. The flat nature of the pressure-volume curve at TLC means that any changes in FVC due to increased strength of the inspiratory muscles are likely to be small, but may be sufficient to be reflected in an improved FEV_1.

A potential confounding influence that may explain why there were no changes in baseline R_{rs} is the effect of airway carbon dioxide (CO_2). The results of Chapter 5 demonstrated that airway CO_2 modulates the effect of multiple loaded breaths on

R_{rs}. It was shown that when normocapnia was maintained, increases in R_{rs} were attenuated beyond that seen when hypocapnia occurred. Consequently, the impact that the modulating role airway CO_2 may have on the efficacy of IMT as a long term intervention is unclear. Patients with asthma often exhibit $P_{ET}CO_2$ at the lower end of the normal range (36-42 mmHg) and exhibit significant bronchoconstriction when hypocapnia is induced by voluntary hyperventilation (van den Elshout *et al.*, 1991; Osborne *et al.*, 2000). Also, when hypercapnia is induced by re-breathing a reversal of the bronchoconstriction is seen (van den Elshout *et al.*, 1991). The mechanism responsible for hypocapnia induced bronchoconstriction is unknown but may be related to changes either within or external to the ASM (Bruton & Holgate, 2005). Potential factors include neural reflexes, increased blood vessel calibre, stimulation of mediator release, as well as via a direct effect on the ASM (Bruton & Holgate, 2005). It is thought that for hypocapnia to have a direct effect on ASM, $P_{ET}CO_2$ needs to be lower than 15 mmHg (Sterling, 1968). Consequently, as the protocol used in Chapter 5 was unlikely to lower $P_{ET}CO_2$ to this level, it is likely that any acute bronchoconstriction seen in response to IMT was due to a hypocapnia induced reflex, mediated by an increase in cholinergic nerve activity (Canning & Fischer, 2001). The reversible nature of hypocapnia induced bronchoconstriction means that baseline measurement of R_{rs} would be unaffected in this study (van den Elshout *et al.*, 1991). However, it is uncertain whether hypocapnia induced bronchoconstriction may have confounded the hypothesised stretching of ASM during the individual bouts of IMT.

Perception of breathing effort

In agreement with previous studies, 6 wks IMT was associated with a reduction in the perception of breathing effort to a loaded breathing task (Weiner *et al.*, 2000b; Covey *et al.*, 2001). The respiratory muscles, like other skeletal muscles, are trainable, as inferred by the 28% improvement in MIP in this study. The mechanisms by which IMT exerts its effect on perception of breathing effort are unclear, but likely to involve a reduced motor drive to the inspiratory muscles. IMT is associated with a 15-22 % reduction in motor drive as assessed by mouth occlusion pressure at 0.1 s ($P_{0.1}$; Huang *et al.*, 2003, 2009). In addition, the minimum stimulus intensity required to evoke a cognitive awareness of increased breathing effort (detection threshold), is also reduced after IMT (Huang *et al.*, 2009). The lowered detection threshold is, however, unique to the mode of IMT used in that pressure-threshold training does not modulate the detection of resistive loads (Huang *et al.*, 2009). Perception of breathing effort is also known to increase in proportion to increases in central drive ($P_{0.1}$ and $\dot{V}_E$) during exercise (Mador & Acevedo, 1991b; Mador & Acevedo, 1991a). The evidence presented above makes it reasonable to propose that an important factor contributing to the IMT-related improvements in perception of breathing effort in this study are related to the influence of IMT upon motor drive to the inspiratory muscles. The strength of the relationship between changes in MIP and perception of breathing effort was strongest at the lowest load and weakest at the highest load (Figure 6.5.). After a period of IMT significantly greater (more negative) mouth pressures can be generated before a load is perceived (Huang *et al.*, 2009). The participants

in this study had a relatively modest increase in MIP (28%) that may explain why the relationship was weaker at the higher threshold load.

It is worth noting that a degree of caution should be used when prescribing IMT to asthma patients with the aim of reducing perception of breathing effort. Although perception of breathing effort is associated with a reduced quality of life it also acts as an important cue to impending asthma attacks and motivates patients to seek medical intervention. In this regard, attenuating an important cue used by patients with severe asthma and/or an already blunted perception of breathing effort, may predispose them to potentially fatal attacks (Kikuchi *et al.*, 1994). With this in mind it is advised that IMT is a safe intervention only in patients with mild/moderate asthma. Other authors support this stance (Weiner *et al.*, 2000a).

In summary, this study has demonstrated that the inherent between day variability in baseline airway resistance is of a magnitude that is likely to mask any potential improvements that may be attributed to IMT. In addition, in contrast to previous studies (Weiner *et al.*, 1992; McConnell *et al.*, 1998), this study also failed to identify improvements in any of the lung function variables measured. The duration of the IMT intervention and the influence of airway CO_2 may have confounded this aspect of the study and need to be considered in any future work in this area. In agreement with previous studies, IMT resulted in improvements in perception of breathing effort across a wide range of threshold loads that were most likely due to a reduction in inspiratory motor outflow concomitant with an

increase in inspiratory muscle strength. Further studies are needed to elucidate the effect of chronic IMT on airway resistance in patient populations.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS FOR FUTURE RESEARCH

7.1. INTRODUCTION

The aim of this chapter is to summarise the main findings of the thesis, discuss them in relation to current knowledge, and to draw conclusions regarding any implications arising from the data. Recommendations for future research will also be provided.

7.2. SUMMARY OF THESIS OBJECTIVES

The principal aim of this thesis was to determine the influence of pressure-threshold inspiratory muscle training (IMT) upon upper and lower airway function; the focus of the thesis being upon the mechanical properties of the structures involved. A number of objectives were stated in the introduction and these are outlined below.

1. To determine whether primary upper airway dilator muscles are activated by an acute bout of inspiratory pressure-threshold IMT.

2. To examine the effect of IMT on upper airway narrowing during loaded breathing.

3. To determine the acute effect of deep inhalation and a range of inspiratory pressure-threshold loads upon respiratory system resistance in people with moderate, stable asthma.

4. To investigate the effects of IMT upon spirometry, baseline respiratory system resistance, and response to deep inhalation in people with moderate, stable asthma.

7.3. SUMMARY OF MAIN FINDINGS

7.3.1. Increased Activation of Lingual Muscles in Response to Acute Inspiratory Pressure-Threshold Loading in Healthy Human Beings: An MRI Study

The aims of Chapter 3 were to determine whether two upper airway dilator muscles, genioglossus (GG) and geniohyoid (GH), were activated in response to an acute bout of pressure-threshold IMT. Changes in the T_2 relaxation time of muscle water were used to identify muscle activation after an acute bout of IMT at 60% MIP, undertaken to task failure (~ 5 min). Immediately after the acute bout of IMT, T_2 values were elevated above baseline for GG ($P < 0.001$) and GH ($P < 0.001$). Thus, data presented in Chapter 3 of this thesis is new and supports the use of IMT to activate muscles of the UA. In addition, the data also provide a rationale for the use of IMT as a tool to strengthen the muscles of the UA.

7.3.2. Effect of Six Weeks Inspiratory Pressure-Threshold Loading on the Upper Airway Response to Loaded Breathing: An MRI Study

The aims of Chapter 4 were to quantify the relationship between inspiratory loading and the associated narrowing of the upper airway (UA). Further, the effect of 6 weeks pressure-threshold IMT on this relationship was examined. The main findings of Chapter 4 were that despite a substantial reduction in airway cross-sectional area at an inspiratory load of 10% MIP, no further reductions in CSA were seen at higher loads (30 and 50% MIP). Further, most of the reduction in CSA was due to narrowing of the lateral pharyngeal walls. No effect was found of IMT upon either resting measurements of UA dimensions, or those during

inspiratory loading. This was interpreted as an indication that biological variability of upper airway calibre makes magnetic resonance imaging (MRI) unsuitable for examining chronic luminal changes.

7.3.3. Acute Effect of Inspiratory-Pressure Threshold Loading upon Airway Resistance in People with Asthma

The purpose of Chapter 5 was to investigate the acute effect of various inspiratory pressure-threshold loads, as well as an unloaded deep inhalation (DI), on respiratory system resistance (R_{rs}) in people with moderate, stable asthma. The main finding was that the increase in R_{rs} seen after an unloaded DI was attenuated when participants breathed either single or multiple breaths against a pressure-threshold load equivalent to 50% MIP. Further, the effect seen after multiple breaths was enhanced when airway carbon dioxide was maintained within normocapnic limits. The findings from this chapter are new and provide a rationale for the use of pressure-threshold IMT in modulating the airway smooth muscle in people with moderate, stable asthma.

7.3.4. Effect of Six Weeks Inspiratory Pressure-Threshold Loading upon Airway Function and Sensation of Breathing Effort in People with Asthma

The aim of Chapter 6 was to build on the findings reported in the previous chapter and to determine the effect of a 6-week period of pressure-threshold IMT on various indices of airway function in people with moderate, stable asthma. In addition, the response to DI and perception of breathing effort to incremental threshold loading was also evaluated before and after the 6-week training period.

Six weeks of IMT did not alter resting airway function or the response to DI. Perception of breathing effort was lowered across all loads compared to baseline and placebo.

7.4. DISCUSSION AND IMPLICATIONS OF MAIN FINDINGS

7.4.1. Inspiratory Muscle Training and Upper Airway Muscle Function

Results from this thesis have demonstrated that the GG and GH are both activated during an acute, fatiguing bout, of pressure-threshold IMT. The functional significance of chronic IMT on UA function is unclear and the following section will attempt to discuss this in the context of previous research.

Increased activation of the UA muscles in response to acute pressure-threshold IMT

The mechanisms by which IMT targets the muscles of the UA are likely the same as the mechanisms that determine UA muscle activity at rest and during hyperpnoea. The integrity of the UA lumen is maintained by rhythmic pre-inspiratory activation of the UA muscles, with EMG activity preceding airflow by approximately 92 ± 34 ms (Strohl *et al.*, 1980). Further, it is known that pressure within the UA also influences the activity of UA muscles, with decreases in UA pressure associated with a concomitant increase in tonic and phasic activity of the GG, which is abolished with application of positive pressure (Mathew *et al.*, 1982). The response to negative pressure is governed by reflex pathways that serve to overcome mechanical loads that may inhibit ventilation, although a plateau in the GG EMG response is seen at pressures below -15 cmH_2O (Horner *et al.*, 1991b). Thus, it would appear that negative pressure within the pharynx acts as a functional load that the UA dilator muscles must overcome to ensure the patency of the UA. Previous research has reported increased activity of the GG in

response to physiological and supra-physiological loads applied using inspiratory resistive loading (IRL) (Malhotra *et al.*, 2000; Pillar *et al.*, 2001).

Pressure-threshold IMT requires the participant to generate a negative pressure of sufficient magnitude to overcome the pressure-threshold load. In doing so, a more negative pressure is generated within the UA resulting in an increased mechanical load that the dilator muscles of the UA must overcome to prevent airway collapse. It was demonstrated in Chapter 4 that the extent of UA collapse did not increase with negative pressures in excess of -10 cmH_2O. All of the UA collapse occurred in the non-muscular lateral walls, with airway patency maintained by preservation of the anteroposterior axis, presumably by increased GG and GH activity. It is therefore possible that, since GG EMG activity plateaus at loads in excess of -15 cmH_2O (Horner *et al.*, 1991b), the preservation of the anteroposterior axis (and UA integrity) during IMT may require maximal activation of the GG and GH, commencing at relatively low inspiratory loads (-10 cmH_2O).

The data presented in Chapter 3 is the first to demonstrate the effect of acute IMT on the activation of the UA muscles GG and GH. In addition, this was the first time increases in the transverse relaxation time of muscle water (T_2) were used to report the activity of these muscles. Increased T_2 was reported for the GG and GH within 5 min post-IMT to task failure at 60% maximum inspiratory mouth pressure (MIP). The increase in T_2 seen in the GG and GH was likely due to work-related changes in their metabolic activity due to maximal activation in overcoming the pressure-threshold load. Given that GG EMG plateaus at loads in

excess of -15 cmH_2O, it is perhaps unsurprising that increased activity of the GG and GH, after an acute bout of pressure-threshold IMT at 60% MIP was shown to occur. However, as stated this finding is new and supports the use of pressure-threshold IMT to activate the GG and GH. Further, the evidence provides a new and clear rationale to investigate the role of chronic IMT in modulating UA structure and function.

Effect of 6 weeks IMT on UA function

The theoretical underpinning for the rationale of training the muscles of the UA to improve their function is provided by a number of studies, as well as by the well-established responses of skeletal muscles to chronic training stimuli. New evidence to support the use of pressure-threshold IMT to activate the UA muscles, specifically the GG and GH, was provided in Chapter 3.

The muscles of the UA are skeletal and consequently respond to the same adaptive processes as other skeletal muscle. In response to overload skeletal muscle adapts through increased protein synthesis that results in hypertrophy and an increased ability to produce force (Harridge, 2007). Little data exists on the characteristics of the UA muscles in healthy people, as most of the work in this area has looked at pathological states such as obstructive sleep apnoea syndrome (OSAS) and snoring. However, in one study that compared OSAS patients and healthy individuals the authors reported significantly greater muscle tissue in the musculus uvulae in OSAS patients (Stauffer *et al.*, 1989). It has also been reported that, compared to snorers, the musculus uvulae of patients with OSAS have a

greater tension generating capacity, increased anaerobic enzyme activity, and a tendency toward increased fatigability (Series *et al.*, 1995). Further, an increase in the prevalence of myosin heavy chain (MHC) Type IIa muscle fibres and increased fatigability have also been reported in the GG of OSAS patients (Series *et al.*, 1996; Carrera *et al.*, 1999). Thus, there is a body of evidence that supports the notion that the UA muscles respond and adapt to increased resistive loading; at least in pathological states (Series, 2002). However, any training adaptation in skeletal muscle is highly specific to the stimulus received. In the case of OSAS the stimulus consists of intermittent, quasi-isometric, near maximal activations that lead to shifts toward fast-twitch, more fatigue prone fibre types (Series, 2002). Whilst contractility may be preserved, the ability to sustain force generation with repeated activations is likely to be diminished. Consequently, a progressive loss of force generation is likely, due to an increased propensity to fatigue. The adaptations experienced by patients with OSAS would, therefore, appear to represent a maladaptive response that sacrifices fatigue resistance in favour of increased force generation (Kimoff, 2007). By applying a quantified load that targets both the strength and endurance characteristics of the UA muscles an improvement in airway function may result that, in turn, could improve the ability of UA muscles to maintain the integrity of the airway lumen. Recent evidence suggests that training the UA muscles using oropharyngeal exercises significantly reduces OSAS symptoms and severity (Guimaraes *et al.*, 2009). It was suggested that the mechanism most likely responsible for the reported outcomes was increased tongue protrusion force (Steele, 2009). Interestingly, a direct association has recently been described between tongue

protrusion force and maximal inspiratory mouth pressure (MIP), which supports the rationale that strengthening the GG (the primary tongue protruder muscle) using pressure-threshold IMT, may provide a potent stimulus for UA remodelling (Shepherd *et al.,* 2006). The suitability of IMT for activating the GG was reported in Chapter 3 and this, coupled with the increases in MIP reported in Chapter 4, suggests that a training stimulus was provided to the UA muscles during the 6 week IMT intervention. An attempt was made to quantify the effect of IMT on UA structure and function using MRI with images of the UA acquired pre- and post-IMT under conditions of rest and during various inspiratory loads. A number of measurements were made in the axial and sagittal plane to determine whether IMT was able to induce structural and functional changes to the UA. Unfortunately, the inherent biological variability of the UA meant that any improvements, should they have occurred, were not detectable using MRI. Whilst it was not possible to directly quantify the effectiveness of IMT on UA function, either by MRI or the measurement of tongue protrusion force, its efficacy may be inferred. The association between tongue protrusion force and MIP, and the increase in MIP after the IMT intervention, make it highly likely that improvements in tongue protrusion force were present after IMT. If this was indeed the case, it is likely that this had a positive structural and functional influence on the UA, albeit one that was not possible to quantify.

Training the muscles of the UA using IMT may also be applicable to healthy populations. Spengler (2002) referred to unpublished observations from her laboratory of increased total airway resistance after prolonged normocapnic

hyperpnoea. It was suggested that the increase in airway resistance may have been due to fatigue of the UA muscles (Spengler, 2002). The fatigability of the GG was determined by Scardella *et al*. (1993). GG endurance was assessed at 80% of its maximal force generating capacity pre- and post- IRL. Participants breathed for ten minutes against an IRL of 40 $cmH_2O \cdot L^{-1} \cdot s^{-1}$ that was sufficient for the GG to generate approximately 35% of its maximal force. The EMG activation level at this resistance was approximately 20% of that achieved during the determination of maximal force. Compared to control GG endurance decreased by approximately 50% after IRL (Scardella *et al.*, 1993). During maximal upright exercise, GG EMG activity has been shown to average 40% of its maximal activity, which would equate to approximately 55% of its maximal force generating capacity (Scardella *et al.*, 1993; Williams *et al.*, 2000). Scardella *et al*. (1993) suggest that 30% of maximal force represents the point at which GG force generation could, theoretically, be sustained indefinitely. Any level of force that exceeds this, such as with high intensity exercise, could impose sufficient demands on the GG to cause fatigue. Importantly, the 30% of maximal force that is suggested as the fatigue threshold of the GG is lower than that of the diaphragm whose maximal sustainable force generating capacity is reported to be between 40 and 50% of maximum force (Roussos & Macklem, 1977). The evidence suggests that GG endurance could be reduced by short-term activation that is insufficient to fatigue the thoracic respiratory muscle (Scardella *et al.*, 1993). The consequences of a fatiguing GG may be an increase in upper airway resistance as well as an increase in the work of breathing. In addition, an increase in respiratory effort sensation for a given level of ventilation has also been suggested (Spengler,

2002). It is known that in rats exercise hyperpnoea is responsible for a fast to slow shift in MHC content in the UA muscles digastric and sternohyoid, as well as increased oxidative capacity (Vincent *et al.*, 2002). The data presented above supports the contention that the hyperpnoea of exercise also provides a functional load to the UA of human beings, which could induce a training response in the UA muscles. It appears likely, therefore, that exercise of sufficient magnitude should induce an UA training response similar to that seen in rodents. Further, it is feasible that specific training of the UA dilator muscles may supplement the exercise-induced response of the UA, which may enhance their function during exercise. Potential benefits of specific UA dilator muscle training include enhanced resistance of any fatigue associated with exercise hyperpnoea and an associated attenuation of increases in UA resistance. In addition, a reduction in the work of breathing for a given level of ventilation and a reduced perception of breathing effort may also occur.

Another manifestation of exercise induced UA fatigue in athletes may be related to the inspiratory stridor associated with repeated bouts of high intensity exercise. High intensity exercise typically demands high ventilation rates and accordingly the prevalence of stridor is higher in the athletic, compared to the general, population (Rundell & Spiering, 2003). Stridor is often associated with paradoxical vocal fold motion (PVFM), which involves the vocal folds paradoxically adducting during inspiration leading to a pronounced and often loud wheeze. The aetiology of PVFM is unclear but the association of the condition with high levels of ventilation, and consequently, a high work of breathing,

support the contention that fatigue of the posterior crico-arytenoid (PCA) muscle may be a potential mechanism. A number of case study reports have shown that IMT is effective in treating stridor in athletes (Ruddy *et al.*, 2004; Mathers-Schmidt & Brilla, 2005; Dickinson *et al.*, 2007), although the mechanism behind any improvement in symptoms is unclear. A potential mechanism, however, may be related to a combined training effect between the PCA and the diaphragm that results in enhanced function of the PCA muscle (Baker *et al.*, 2003b). Specifically, post-IMT enhancements in the size of the glottal aperture during inspiration could reduce airway resistance, work of breathing and perception of breathing effort.

7.4.2. Recommendations for Future Research

Future studies should first establish whether IMT results in improvements in tongue protrusion force, as it appears likely that this provides an objective measure for determining improvements in UA muscle function (Shepherd *et al.*, 2006; Steele, 2009). Depending on the outcomes, additional studies should aim to establish optimum training loads as well as the functional significance of any improvements in the strength and endurance characteristics of the GG. For example, sleep studies in OSAS patients could determine the effectiveness of IMT in improving sleep quality, the incidence of sleep disturbance, and frequency of apnoeic events.

Studies in healthy groups should determine the effect of exercise on the strength and endurance characteristics of the GG; as well as quantifying the influence of

exercise upon these characteristics. Surface EMG may also provide a method to quantify the effect of prolonged or high intensity exercise on GG function. Further studies would need to determine the implications of exercise-induced fatigue of the UA musculature. If exercise induced fatigue of the UA is identified, future studies might also examine the efficacy of IMT as a means of attenuating this fatigue, and the impact of this upon breathing mechanics.

7.4.3. Inspiratory Muscle Training and Lower Airway Function

Results presented in this thesis have demonstrated that an acute bout of IMT at 50% MIP, performed as single or multiple breaths mitigates the increase in R_{rs} seen after a single DI in participants with moderate, stable asthma. The functional significance of chronic IMT on R_{rs} is unclear and the following section will attempt to discuss this in the context of previous research.

Acute response of the airway to a single bout of pressure-threshold IMT

People with asthma typically demonstrate an increase in airway resistance in response to a DI (Kapsali *et al.*, 2000; Brown *et al.*, 2001), and this was confirmed in Chapter 5 of this thesis. The mechanism(s) under-pinning this response is thought to reside within the airway smooth muscle, which is stiffer and less compliant than that of healthy people. Consequently, the airway of a person with asthma is less distensible and less responsive to the stretching influence of lung inflation. Further, the extent of airway re-constriction, seen in people with asthma, is greater in those who are less able to dilate with a DI (Salome *et al.*, 2003). In the healthy person a DI acts as a potent bronchodilator that is able to overcome

pharmacologically induced bronchoconstriction (Nadel & Tierney, 1961; Lim *et al.*, 1987; Brown & Mitzner, 1996). In patients with asthma, the mechanism responsible for the bronchodilating effect of DI fails and, in some instances, worsens the degree of bronchoconstriction (Lim *et al.*, 1987).

In Chapter 5, it was reported that single and multiple breaths against a pressure-threshold load of 50% MIP attenuated the increase in R_{rs} seen after DI. It was suggested that this was likely related to the effect of greater transluminal pressure, supplementing the stretch applied to the airway by volume related parenchymal traction on the airway smooth muscle (ASM). This suggestion is supported by the study of Duggan *et al.*, (1990) who dissociated the effects of lung volume and transpulmonary pressure using chest wall strapping (CWS). These authors demonstrated that similar levels of bronchodilation could be achieved at a lower lung volume with the higher transpulmonary pressures achieved by CWS (Duggan *et al.*, 1990). A typical bout of IMT is performed at 50% MIP (~ 50-60 cmH_2O) and pressures of this magnitude have been shown to be required to induce bronchodilation in dogs, with pressures less negative than -35 cmH_2O inducing bronchoconstriction (Brown & Mitzner, 2001). The inertial properties of a pressure-threshold load, and the associated intrathoracic gas decompression, may provide an additional mechanism to explain the attenuation of the bronchoconstrictor response to DI.

Another factor that needs to be considered when interpreting the effect of multiple breaths on airway function is the role of airway CO_2. The data presented in

Chapter 5 suggests that airway CO_2 had a confounding influence that modulated the effect of the inspiratory load on the airway. Bruton & Holgate (2005) have previously described the bronchoconstrictive effect of hypocapnia and it would appear that this effect was responsible for attenuating the effectiveness of the 50% MIP inspiratory load. Specifically, when hypocapnia occurred, due to transient hyperventilation, the ability of the inspiratory threshold-load to attenuate the bronchoconstrictive effect of DI was diminished. When hypocapnia was prevented, by re-breathing, the multiple breaths were effective in reducing the bronchoconstrictive effect of DI.

The data presented in Chapter 5 was both confirmatory and new. First, the existing knowledge of the paradoxical response to a DI seen in people with asthma was confirmed. Specifically, participants showed a 15.7 ± 11.0% increase in R_{rs} after a DI to TLC. The new contribution to knowledge presented in Chapter 5 was the observation of an attenuation of the paradoxical response to DI, after both a single loaded breath at 50% MIP and 30 loaded breaths at 50% MIP. As discussed above, the magnitude of the response to multiple breaths appears to be modulated by changes in airway CO_2, which is also a new finding.

The multiple breath protocol was designed to simulate a typical IMT session which, when undertaken over a number of weeks, has been shown to be associated with improvements in forced expiratory flows (Weiner *et al.*, 1992; McConnell *et al.*, 1998). Whilst the acute effect of 30 loaded breaths was no greater than that of one breath, the response to 30 loaded breaths does demonstrate that IMT has the

potential to influence lung function. Consequently, the evidence presented in Chapter 5 provides both a potential explanation for the post-IMT improvements in FEV_1 previously reported, and a new and clear rationale for testing the effect of chronic IMT on airway function in people with asthma.

Effect of 6 weeks IMT on lower airway function

The primary rationale for using chronic IMT to improve lower airway function was provided by the observation that some studies have shown improvements in forced expiratory flows post-IMT (Weiner *et al.*, 1992; McConnell *et al.*, 1998). Further, the data presented in Chapter 5 (discussed above) adds support to this rationale. A potential mechanism for improvements in forced expiratory flow rates post-IMT may be related to the finding that the force generating ability of porcine ASM *in vitro* is inhibited by the application of prior length oscillations over a period of 5 min (Wang *et al.*, 2000). Further, it has also been shown that changes seen in airway properties due to mechanical strain *in vitro* can be induced by physiological interventions *in vivo*. For example, rabbits breathing at a chronically elevated lung volume have airways that are larger and less responsive to methacholine, compared to controls (Xue *et al.*, 2005). Another animal study compared the airways of rabbits subjected to physiological levels of strain with those subjected to no strain (Tepper *et al.*, 2005). The bronchi that had been exposed to chronic strain were less stiff than those not exposed to strain. Further, changes in lumen area due to chronic strain were greatest in the smaller airways, suggesting that these airways are more susceptible to the chronic effects of strain. Tepper and colleagues have suggested that the effects of chronic strain may be

directly due to mechanical forces acting on the ASM (Tepper *et al.*, 2005). Thus, chronic stretching is able to modulate the structural and force generating characteristics of ASM. A recent epidemiological study reported an inverse relationship between weekly exercise and bronchial hyperresponsiveness in 5518 adults (Shaaban *et al.*, 2007). Shaaban *et al.*, (2007) suggested that a potential causative mechanism to explain the finding was that the DIs associated with increased activity may provide a bronchoprotective effect to the airway. Further, it has been suggested that reductions in the amount of periodic stretching of the airway, due to sedentary behaviour, may be a contributing factor for bronchial hyperresponsiveness in children (Hark *et al.*, 2005). Thus, there is a growing body of circumstantial evidence that supports the notion that chronic IMT may be capable of modifying ASM function to reduce resting R_{aw}.

In Chapter 6, the effects of a six-week period of IMT were reported. The influence of IMT upon ASM was assessed by measuring baseline R_{rs}, forced spirometry, and response to DI. This study showed that the between day variability of baseline R_{rs} was of a size likely to mask any potential benefit to airway function, which may be attributed to IMT. Further, contrary to previous studies (Weiner *et al.*, 1992; McConnell *et al.*, 1998), this study found no improvement in any of the lung function variables measured using forced expiratory manoeuvres. Accordingly, it is difficult to determine whether the absence of any effect of IMT upon R_{rs} and DI was due to measurement error, or to the fact that, in this study, IMT failed to influence airway function. It is possible that IMT-induced airway hypocapnia in the present study may have had a confounding influence on R_{rs}.

Differences in the implementation of IMT may go some way to explain the discrepancy, since some authors have recommended a slower breathing pattern during IMT than was used in the present study (Weiner *et al.*, 1992). This would have minimised changes in airway CO_2 and may therefore have permitted the effect of the loading upon ASM to be expressed as an improvement in airway function (Weiner *et al.*, 1992; McConnell *et al.*, 1998).

7.4.4. Recommendations for Future Research

Future research into the acute effect of pressure-threshold IMT on R_{rs} and response to DI should consider using intra-breath measurements similar to that of Jensen *et al*. (2001). These authors tracked R_{rs}, using a modified forced oscillation technique, at baseline and during respiratory manoeuvres in healthy individuals and patients with moderate and severe asthma. The advantages of this method are that the acute response to a DI and/or a loaded breath can be tracked in real time rather than post manoeuvre. This would allow the determination of whether a loaded breath was able to lower R_{rs} to a greater extent than DI alone. The potential bronchoprotective effect of acute loading also warrants further investigation, as this might provide a convenient means of inducing refractoriness prior to exercise.

The chronic effects of IMT on airway function are more difficult to discern and are somewhat dependent on the outcomes of studies into the acute response. However, future studies should incorporate a method that seeks to better standardise the baseline measure of R_{rs}, perhaps by inducing bronchoconstriction using methacholine. Pre- and post-IMT changes in the responsiveness to

methacholine inhalation (PC_{20}) may provide a suitably objective outcome measure to determine chronic changes in R_{rs}. The stability of the DI response to methacholine would need to be established, although its repeatability has been reported as good (Juniper *et al.*, 1978). It is also important that the potential confounding influence of airway hypocapnia during IMT is clarified, as this appears to have the potential to disrupt any beneficial influence of pressure-threshold loading upon ASM.

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APPENDICES

A-1 ETHICAL APPROVAL

School of Sport & Education

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UNIVERSITY
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Middlesex, UB8 3PH, UK
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9th February 2006

Stephen How
School of Sport and Education
Brunel University
Uxbridge
Middlesex
UB8 3PH

Dear Mr How

RE01-06: The perception of Dyspnoea to graded inspiratory efforts in healthy humans.

I write to confirm that the Research Ethics Committee of the School of Sport and Education has considered the application referred to above. We were satisfied that the application meets the ethical requirements of Brunel University. We therefore grant consent to the study and wish you every success with the project.

Yours sincerely

Dr Simon Bradford
Chair, Research Ethics Committee

c.c. Dr Lee Romer

Head of School of Sport & Education
Professor Susan Capel

Brunel
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Heinz Wolff Building,
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Steven How
School of Sport & Education
Brunel University

4th May 2007

Dear Steve

RE19-07 Influence of Inspiratory Muscle Training Upon Airway Resistance, Corticospinal Excitability and Cardiac Vagal Tone in People With Asthma

I write to confirm that the Research Ethics Committee of the School of Sport and Education has considered the application referred to above. We were satisfied that the application meets the ethical requirements of Brunel University. We therefore grant consent to the study and wish you every success with the project.

Yours sincerely

Dr Simon Bradford
Chair of Research Ethics Committee

School of Sport & Education

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9th February 2006

Stephen How
School of Sport and Education
Brunel University
Uxbridge
Middlesex
UB8 3PH

Dear Mr How

RE02-06: Influence of acute insiratory loaded breathing on airway resistance.

I write to confirm that the Research Ethics Committee of the School of Sport and Education has considered the application referred to above. We were satisfied that the application meets the ethical requirements of Brunel University. We therefore grant consent to the study and wish you every success with the project.

Yours sincerely

Dr Simon Bradford
Chair, Research Ethics Committee

c.c. Dr Lee Romer

Lee Romer

From: Simon Bradford
Sent: 11 November 2004 11:43
To: Lee Romer
Subject: RE: Ethics

Lee, members of Research Ethics Committee have been through the paperwork for your project and we are satisfied that everything seems to have been convered in a comprehensive and rigorous way. The only thing that I noticed in the 'Research Participant Information Sheet' on page 2 under 'Are There any Risks', and then under 'Lung function tests' , rather than referring to 'risks' you refer to 'discomforts'. Issue of consistency?

Anyway, Iam assuming that you are happy to proceed on the basis of this e-mail.

Best

Simon

Dr Simon Bradford
Department of Education
Brunel University
300 St Margarets Road
Twickenham
Middx
TW1 1PT
UK

-----Original Message-----
From: Lee Romer
Sent: 09 November 2004 13:23
To: Simon Bradford
Subject: RE: Ethics

OK Simon - sounds like a plan. I look forward to your response re the MRI study...

Regards

Lee

-----Original Message-----
From: Simon Bradford
Sent: 09 November 2004 13:21
To: Lee Romer
Subject: RE: Ethics

Thanks Lee, this clarifies things, thought I'd missed something! as far as i'm aware, each School is required to set up an internal committee as we've done. I will e-mail him and check it out,

I hope to have a response to the MRI thing later this week,

thanks

Simon

Dr Simon Bradford
Department of Education
Brunel University
300 St Margarets Road
Twickenham
Middx

A-2 SAMPLE INFORMED CONSENT FORM

CONSENT FORM

Please tick appropriate box

	YES	NO
Have you read the Research Participant Information Sheet?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Have you received satisfactory answers to all your questions?	☐	☐
Who have you spoken to?……..		
Do you understand that you will not be referred to by name in any report concerning the study?	☐	☐
Do you understand that you are free to withdraw from the study:		
- at any time?	☐	☐
- without having to give a reason for withdrawing?	☐	☐
- *(where relevant)* without affecting your future care?	☐	☐
Do you agree to take part in this study?	☐	☐

Signature of Research Participant……Date……

Name in capitals……

Witness statement

I am satisfied that the above-named has given informed consent.

Witnessed by……..Date:……

Name in capitals:……

A-3 GENERAL HEALTH QUESTIONNAIRE

Pre Test Health Questionnaire

	Yes	No
Has your doctor diagnosed you with any respiratory complaint other than asthma?	☐	☐
Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?	☐	☐
Do you feel pain in your chest when you do physical activity?	☐	☐
In the past month, have you had chest pain when you were not doing physical activity?	☐	☐
Is your doctor currently prescribing drugs (for example, water pills) for blood pressure or a heart condition?	☐	☐
Have you smoked within the last two years?	☐	☐
Have you ever suffered a pneumothorax or perforated ear drum?	☐	☐
Do you know of any other reason why you should not participate in this study?	☐	☐

Signature of Participant_____ **Date**_____

Name in capitals_____

A-4 PERCEPTUAL RATING SCALE

0 – Nothing at all

0.5 – Extremely weak (just noticeable)

1 – Very weak

2 – Weak (light)

3 – Moderate

4 – Somewhat strong

5 – Strong (heavy)

6 –

7 – Very strong

8 –

9 –

10 – Extremely strong (almost max)

- **– Maximal**

What is perceived exertion?

Perceived exertion is a method to determine the intensity of effort, stress or discomfort that is felt during exercise. If you imagine walking from a very slow pace to a very fast pace, or from a low grade to a steep grade in gradual stages, the body experiences a change in sensation that lets you know that effort is increasing.

What do the numbers mean?

If you were resting quietly you would assign a rating of 0 (nothing at all) to describe the breathing sensations that you were experiencing, i.e. no exertion at all, or the lowest exertion imaginable. At the other end of the scale you would assign • (maximal) to the greatest breathing discomfort imaginable. At this end of the scale you can assign any number above 10 to describe your feelings of exertion.

What do I have to do?

When you are asked to describe your feelings of exertion please provide a number from the scale that best reflects the level of breathing discomfort you are experiencing at that moment in time. THERE ARE NO RIGHT OR WRONG ANSWERS.

A-5 MRI SCREENING AND CONSENT FORMS

ROYAL HOLLOWAY, UNIVERSITY OF LONDON - MAGNETIC RESONANCE IMAGING UNIT

INITIAL SCREENING FORM

NAME OF PARTICIPANT ..... Sex: M / F

Date of birth..... Approximate weight in kg..... (one stone is about 6.3 kg)

Please read the following questions CAREFULLY and provide answers. For a very small number of individuals, being scanned can endanger comfort, health or even life. The purpose of these questions is to make sure that you are not such a person.

You have the right to withdraw from the screening and subsequent scanning if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will be held in secure conditions.

Delete as appropriate

1. Have you been fitted with a pacemaker or artificial heart valve? YES/NO
2. Have you any aneurysm clips, shunts or stents in your body or a cochlear implant? YES/NO
3. Have you ever had any metal fragments in your eyes? YES/NO
4. Have you ever had any metal fragments, e.g. shrapnel in any other part of your body? YES/NO
5. Have you any surgically implanted metal in any part of your body, other than dental fillings and crowns (e.g. joint replacement or bone reconstruction) YES/NO
6. Have you ever had any surgery that might have involved metal implants of which you are not aware? YES/NO
7. Do you wear a denture plate or brace with metal in it? YES/NO
8. Do you wear a hearing aid? YES/NO
9. Have you ever suffered from any of: epilepsy, diabetes or thermoregulatory problems? YES/NO
10. Have you ever suffered from any heart disease? YES/NO
11. Is there any possibility that you might be pregnant? YES/NO
12. Have you been sterilised using clips? YES/NO
13. Do you have a contraceptive coil (IUD) installed? YES/NO
14. Are you currently breast-feeding an infant? YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNED..... DATE.....

In the presence of ..... (name) .....(signature)

Address of witness, if not the experimenter:

Please enter here the name and address of your doctor (general practitioner):

ROYAL HOLLOWAY, UNIVERSITY OF LONDON - MAGNETIC RESONANCE IMAGING UNIT

SECOND SCREENING FORM

This form should be completed and signed immediately before your scan, after removal of any jewellery or other metal objects and (if required by the operator) changing your clothes.

NAME OF PARTICIPANT ……

Date of birth……. Sex: M / F

Please read the following questions CAREFULLY and provide answers. For a very small number of individuals, being scanned can endanger comfort, health or even life. The purpose of these questions is to make sure that you are not such a person.

You have the right to withdraw from the screening and subsequent scanning if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will he held in secure conditions.

BEFORE YOU ARE TAKEN THROUGH FOR YOUR SCAN IT IS ESSENTIAL THAT YOU REMOVE **ALL METAL OBJECTS** INCLUDING:-WATCHES, PENS, LOOSE CHANGE, KEYS, HAIR CLIPS, ALL JEWELLERY, BRASSIERES WITH METAL FASTNERS, METALLIC COSMETICS, CHEQUE/CASH POINT CARDS.

Delete as appropriate

1. Are you wearing or carrying any metal items such as those listed above? YES/NO
2. Have your answers to any of the questions in the initial screening form changed?
   (The initial screening form must be shown to you before you answer this question.) YES/NO

Specifically, please confirm:

3. Have you been fitted with a pacemaker, artificial heart valve or cochlear implant? YES/NO
4. Is there any possibility that you might be pregnant? YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNATURE…… DATE……

FOR STAFF USE:

I certify that the initial screening form and the consent form have been completed by the person named above and I have attached them to this form. The volunteer has been given the standard information sheet about MRI experiments, together with any necessary study-specific information, and has been given an opportunity to ask questions. I am satisfied that the volunteer is adequately informed and understands the content of the consent form. I have taken adequate steps to ensure that the volunteer has no ferro-magnetic metal in or on his/her person and I am satisfied that the scan can proceed.

SIGNATURE…… NAME (print) ……..

ROYAL HOLLOWAY, UNIVERSITY OF LONDON - MAGNETIC RESONANCE IMAGING UNIT

CONSENT FORM

NAME OF PARTICIPANT……..……

Please read the following statement carefully and then add your signature. If you have any questions, please ask the person who gave you this form. You are under no pressure to give your consent and you are free to withdraw from the MRI examination at any time.

I agree to participate in an MRI examination conducted for research purposes by

…… (name of operator)

On ……....(name of project).

I understand that the examination is not part of any medical treatment.
I have completed two screening forms and I have been given an opportunity to discuss any issues arising from it.
The nature of the examination has been explained to me and I have had an opportunity to ask questions about it.
I consent to my general practitioner being contacted in the unlikely event that the scan reveals any suspected abnormality.

Signature …… Date……

FOR STAFF USE:

Statement by a witness, who must be either an authorised person or a scientific collaborator who is familiar with the experimental procedure and is able to answer questions about it.

I certify that the above participant signed this form in my presence. I am satisfied that the participant fully understands the statement made and I certify that he/she had adequate opportunity to ask questions about the procedure before signing.

Signature……. Date……

Name ……..

Address of witness (if not an Authorised Person):

A-6 USE OF PLACEBO IN RESEARCH

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USE OF PLACEBO IN RESEARCH

During your participation in this study you were asked to take an inhaler medication for 3 weeks duration. It was explained that this medication was 'a licensed product and is **NOT a trial drug**. It is safe, will not interfere with any existing medication, and has been authorised for use by Brunel University research ethics committee.' Whilst most of these statements were indeed true, the inhaler you took was not an asthma medication, but a placebo. A placebo is a treatment that has no physical or physiological effect but is intended to give the participant the expectation of an effect.

The use of placebo in research is common practice when outcomes measured are dependent upon volitional effort, subject motivation and expectation. During this study you were asked to rate the level of breathlessness you felt when breathing against various resistances. It would have been reasonable for you to expect that you would have felt less breathless after a period of inspiratory muscle training (IMT) and this may have been reflected in how you rated your breathlessness; therefore, it was appropriate in this study to employ the use of a placebo. The placebo you took was a standard metered dose inhaler containing only propellant (no drug). The intention was for the placebo to generate an expectation in you similar to that which you would expect after inspiratory muscle training. This enabled us to compare the effects of the real treatment (IMT) with the placebo whilst taking into account the expectation you may have had of an improvement in your level of breathlessness.

If you have any concerns regarding this please do not hesitate to contact me.

Thank-you once again for volunteering to take part in this study

Stephen C. How BSc (Hons), MSc

A-7 ARTICLES PUBLISHED DURING THE PREPARATION OF THIS THESIS

How, S.C., Romer, L.M., & McConnell, A.K. (2009). Acute effects of inspiratory pressure-threshold loading upon airway resistance in people with asthma. *Respiratory Physiology and Neurobiology*. **166,** 159-163 http://www.ncbi.nlm.nih.gov/pubmed/19442932

How, S.C., McConnell, A.K., Taylor, B.J., & Romer, L.M. (2007). Acute and chronic responses of the upper airway to inspiratory loading in healthy awake humans: an MRI study. *Respiratory Physiology and Neurobiology*. **157**, 270-280 http://www.ncbi.nlm.nih.gov/pubmed/17341450

Taylor, B.J., **How, S.C.**, & Romer, L.M. (2006). Exercise-induced abdominal muscle fatigue in healthy humans. *Journal of Applied Physiology*. **100**, 1554-1562 http://www.ncbi.nlm.nih.gov/pubmed/16424068